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OBSERVATIONS ON THE PERICAPILLARY CELLS IN THE MESENTERIES OF RABBITS

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ONE PLATE (TWO FIGURES)

This is a report of the reaction to electrical stimulation and the staining reaction with Janus green of pericapillary cells in the mesentery of the rabbit.

METHOD

In the first series, the rabbits were anaesthetized with ether, the omentum or mesentery of the small intestine exposed on a warm stage and observed with a microscope. Stimulation was done with metal electrodes and induction coil. Then the animal was perfused with salt solution, then Janus green, 1:10,000 in salt solution. The staining was stopped by perfusion with 5 per cent ammonium molybdate.

In the second series, the rabbits were decapitated, carotids and jugulars clamped, given artificial respiration, and the rest of the procedure carried out as in the first series.

OBSERVATIONS

The contractility of pericapillary cells in animals lightly anaesthetized with ether

Ten animals in this series were tested. The amount of ether used was the minimum which would keep the animal quiet. Particular care was used in making the necessary exposure of omentum or mesentery. It was kept moist with warm physiological saline solution on a copper warm-stage.

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The observed reactions of vessels in the mesentery were quite constant.

When first observed, the blood would be moving so fast that individual corpuscles could not be seen. However, the outlines of arteries, arterioles, capillaries, venules, and veins would be clearly distinguished and measured (optical section) with an eye-piece micrometer. The circulation was continued without noticeable change for a long time, two to six hours after the abdomen was opened.

Numerous fields were studied in which the circulation from artery to capillary to vein could be seen in one (16-mm. objective × 15x ocular) field. Capillaries anastomosed freely into an extensive network in the mesentery. In the omentum this network was relatively scant.

Stimulation with electrodes close to, but separated from, the vessel by peritoneum, was carried out. Slight stimulation had no noticeable effect on any of the vessels.

Strong stimulation on an artery, central to a particular capillary bed, would cause, first, a slowing, then a cessation of flow through artery, capillary, and vein. The period of slowing, usually about twenty seconds, gave a good view of corpuscles making the trip through the capillary bed. There seemed to be an abundance of plasma as the capillaries usually had a column of corpuscles. At each pulsation a few corpuscles would enter the capillary. It was possible to see a synchronous pulsation in arteriole, capillary, and vein. When the column of corpuscles stopped, there was no evidence of local constriction in the capillary bed. When stimulation ceased, the column of blood flowed toward the arteriole, then reversed to the usual direction, and in one to three seconds was going at high speed again. When flow through capillaries stopped spontaneously it could be started again by stimulation of the artery, but not by stimulation of pericapillary cells. Stimulation of pericapillary cells did not stop the flow, nor cause any constriction in the region of these cells. Increasing the amount of ether given the animal would stop the flow without apparent change in outline of the

capillaries. On studying a small artery at a point where branches are given off, one could see a continuous column of corpuscles driven into one branch while into another of the same diameter a few corpuscles would be driven followed by clear plasma. This could be traced into capillaries. The diameter of the capillaries remained unchanged. Immediately after vessels, 8 μ in diameter, branched off arterioles, there was a slight, though well-marked, constriction noticeable during stimulation of the arteriole. This constriction did not stop the flow completely.

The contractility of pericapillary cells in decapitated animals with carotids clamped and artificial respiration

The flow through the capillary bed in mesentery or omentum was not as rapid as in etherized animals. Slight stimulation had no noticeable effect on any of the vessels. Strong stimulation caused constriction of the arterioles in five to ten seconds. There was marked constriction in the arterioles just after branching off a small artery. Stimulation of an artery caused increased flow for a second or two, then the flow stopped. Stimulation of an arteriole would cause a sharp local constriction in the vessel. Stimulation of the same degree applied to cells around capillaries in the network away from definitely contracting arterioles had no noticeable effect on the flow of blood or the outline of the vessel.

Very strong stimulation around capillary loops stopped the flow of blood and made the outline of the capillary tube irregular. These vessels did not resume circulation or previous outline after stimulation stopped.

The staining reactions of pericapillary cells with Janus green

After the vessels had been stimulated and the arterioles and arteries observed to contract, the animal was perfused, via aorta, with salt solution to wash out the corpuscles and then with Janus green, 1:10,000 in physiological saline solution. When one could see the corpuscles replaced by clear solution, the Janus green was started. Endothelium became

bluish and nuclei quite evident. Smooth-muscle fibers containing myofibrils could be seen on arteries and on arterioles.

On vessels (8 μ in diameter) branching from arterioles a few smooth-muscle fibers could be seen. These areas constricted when stimulated. Constriction would make the lumen so small that red corpuscles would elongate to pass through. No structures which could be called myofibrils were seen in following the vessels peripherally through the capillary network. Preparations were fixed with ammonium molybdate in various stages of staining. The adventitial cells were not stained in preparations which showed myofibrils in smooth muscle. In other preparations in which histiocytes showed numerous granules the adventitial cells were bluish in color without granules (fig. 1). The adventitial-cell processes were arranged in longitudinal relation to the vessels and did not encircle the vessels. Folds in the endothelium in some preparations did encircle the vessels (fig. 2).

DISCUSSION

The presence of contractile cells around capillaries, which contain myofibrils, has been demonstrated in the nictitating membrane of frogs by Bensley and Vimtrup ('28).

Electrical stimulation and progressive staining with Janus green might give the same results when applied to mammalian tissues if the structural components are similar.

Local constriction or indentation of mesenteric capillaries at their origin in rabbits was reported by Tannenberg ('25). The changes were noted after the application of heat or cold and were considered due to the contraction of adventitial cells. Presence of myofibrils around small vessels on the arteriole side of the network in the author's preparations would lead him to classify these vessels as arterioles.

In contrast with the active contraction of naked endothelium in amphibia, Clark and Clark ('32) believe that capillary contraction is rare and quite negligible in effect on mammalian circulation. The work of Clark, Sandison, and others, with tissue chambers, indicates that active contraction takes

place only in the arterioles and arteries, while capillaries and venules have tonicity and elasticity. My observation on flow in the capillary network can be explained logically on this basis, as there was no change in capillary outline from pericapillary stimulation until the tissue had been subjected locally to strong faradic stimulation and afterward failed to respond at all.

CONCLUSIONS

1. Stimulation of cells adjacent to capillaries did not alter the flow of blood or the outline of the capillary unless the current was strong enough to cause local death of tissue.
2. Myofibrils were not demonstrated in the adventitial cells adjacent to the capillaries.

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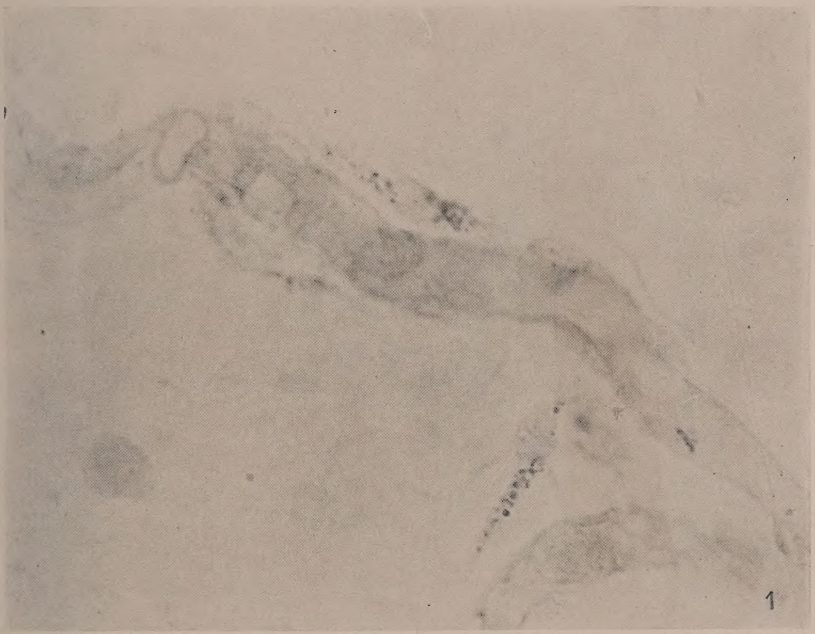
PLATE 1

EXPLANATION OF FIGURES

1 Capillary in mesentery of rabbit. Vital stain with Janus green. Adventitial cell shows no myofibrils. Histiocytes show numerous granules. × 990.

2 Capillary in mesentery of rabbit. Vital stain with Janus green. Shows folds of endothelium which encircle the lumen. × 990.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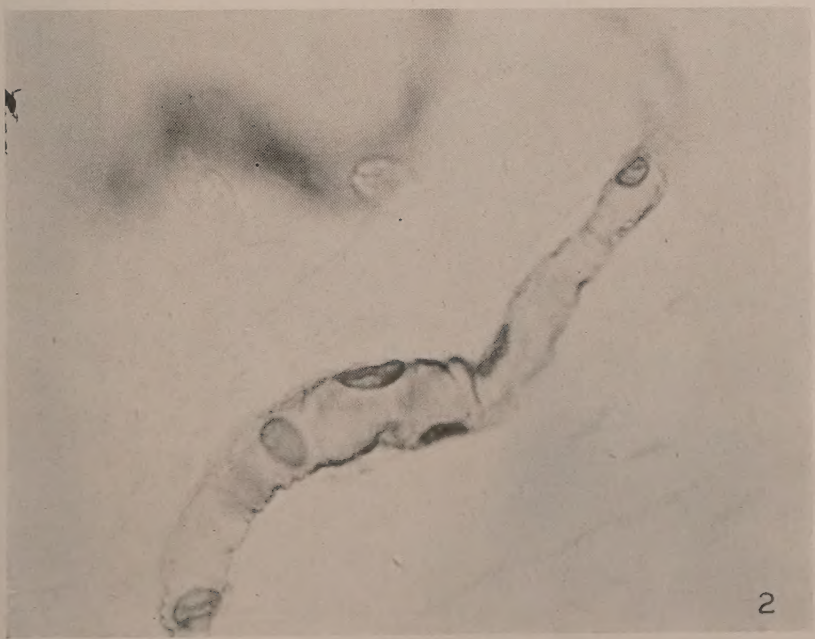
2

ON THE ARRANGEMENT OF THE SUPERFICIAL VEINS OF THE CUBITAL FOSSA IN AMERICAN WHITE AND AMERICAN NEGRO MALES

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ONE FIGURE

The frequent variation in the arrangement of the superficial veins of the cubital fossa has been recognized by both anatomists and clinicians. The arrangement of these veins may be studied by dissection or by placing a tourniquet around the arm of an individual and then observing the pattern resulting as the veins become engorged. The latter method has been employed by Berry and Newton ('08) in British (whites), by Lassila ('27) in Finns, and by Okamoto ('22) in the Japanese. The patterns shown in the illustrations of these veins in the modern text books and atlases of human anatomy are by no means identical.

The present report includes further observations on the cubital veins with respect to the relative frequency of the different forms of arrangement and presents comparison of these forms between American white and American negro males together with other comparisons.

The material consists of 122 cadavers, of which ninety-two were dissected by students during the years 1928 to 1931, inclusive, and thirty were dissected by the author. Sixty bodies were American white males and sixty-two were American negro males, and on each body the veins on both the right and left sides were examined. Thus, 244 observations were made. The method employed was that described by Terry ('29), the essential points of which are that each student

dissection is made under direction and each observation is verified by an instructor.[1]

The types of arrangement of the superficial veins of the cubital fossa are shown in the accompanying diagrams (fig. 1). The occurrence of these types in per cent is shown in table 1. The results may be summarized as follows:

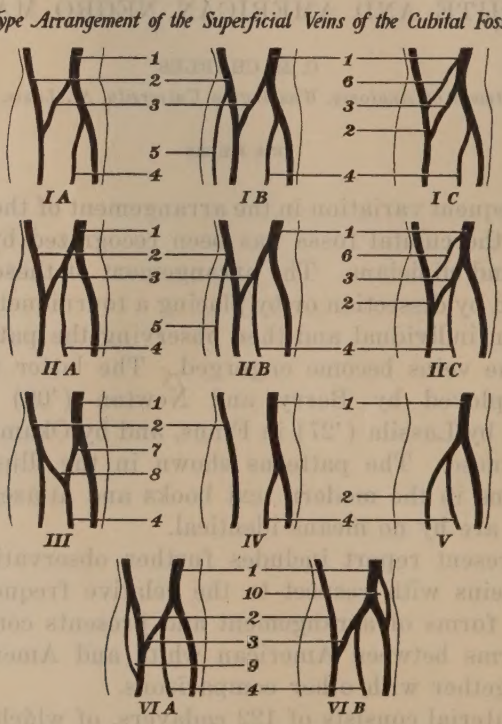

Fig. 1 *1.* V. basilica. *2.* V. cephalica. *3.* V. mediana cubiti. *4.* V. mediana antibrachii. *5.* V. cephalica accessoria. *6.* V. mediana cubiti accessoria. *7.* V. mediana basilica. 8. V. mediana cephalica. 9. 'Inselbildung' v. cephalica. *10.* 'Inselbildung' v. basilica.

[1] Students assisting in this investigation were Messrs. A. M. Boyd, H. C. Shapiro, P. O. Hageman, S. F. Hampton, E. H. Ames, and W. A. Stephens. The author wishes to express his appreciation of the assistance they gave in this study.

TABLE 1

Percentage distribution of types of arrangement of superficial veins of the cubital fossa

TYPE	WHITE MALE		NEGRO MALE		RIGHT AND LEFT		WHITE AND NEGRO		WHITE AND NEGRO	JAPANESE MALE			CAUCASIAN (BRITISH) MALE
	Right (60)	Left (60)	Right (62)	Left (62)	White male (120)	Negro male (124)	Right (122)	Left (122)	Right and left (244)	Right (100)	Left (100)	Right and left (200)	Right and left (300)
I A	15.0	13.3	16.1	30.6	14.2	23.4	15.6	22.1	18.9	Okamoto, K.			Berry, R. J. A., and Newton, H. A. S.
I B	8.3	1.7	9.7	6.5	5.0	8.1	9.0	4.1	6.6				
I C	3.3	1.7	1.6	0.0	2.5	0.8	2.5	0.8	1.6				
I	26.7	16.7	27.4	37.1	21.7	32.3	27.0	27.0	27.1				
II A	28.3	43.3	29.0	19.4	35.8	24.2	28.7	31.1	29.9				
II B	13.3	8.3	16.1	12.9	10.8	14.5	14.8	10.7	12.7				
II C	1.7	1.7	0.0	0.0	1.7	0.0	0.8	0.8	0.8				
II	43.3	53.3	45.2	32.3	48.3	38.7	44.3	42.6	43.4				
I and II	70.0	70.0	72.6	69.4	70.0	71.0	71.3	69.7	70.5			84.5	83
III	28.3	26.7	12.9	11.3	27.5	12.1	20.5	18.9	19.7			1	16
IV	0.0	3.3	14.5	14.5	1.7	14.5	7.4	9.0	8.2	11	18	14.5	0.7
V	1.7	0.0	0.0	4.8	0.8	2.4	0.8	2.5	1.6				0.3
Termination v. mediana antibrachii: Inselbildung v. cephalica	16.7	15.0	21.0	9.7	15.8	15.3	18.9	12.3	15.6	31	23	27	16
Termination v. mediana antibrachii: Inselbildung v. basilica	3.3	1.7	3.2	1.6	2.5	2.4	3.3	1.6	2.5	2	0	1	
Termination v. mediana antibrachii: V. cephalica accessoria	21.7	10.0	25.8	19.4	15.0	22.6	23.8	14.8	19.3	47	39	43	66
Termination v. mediana antibrachii: V. basilica	26.7	20.0	41.9	51.6	23.3	46.8	34.4	36.1	35.2	44	44	44	42
Termination v. mediana antibrachii: V. mediana cubiti	45.0	53.3	45.2	37.1	49.2	41.1	45.1	45.1	45.0	56	53	54.5	43
Termination v. mediana antibrachii: Vv. mediana basilica et mediana cephalica	28.3	26.7	12.9	11.3	27.5	12.1	20.5	18.9	19.7	0	2	1	13
Termination v. mediana antibrachii: Reduplication of v. mediana cubiti	5.0	3.3	1.6	0.0	4.2	0.8	3.3	1.6	2.5	7	10	2.5	4

1. The arrangement most frequently observed (about 70 per cent) was that in which the two longitudinally directed veins, vv. cephalica and basilica, were connected by the v. mediana cubiti. Okamoto ('31) found this type to be present in 84.5 per cent of the arms of Japanese males examined, while Berry and Newton ('08) reported an incidence of 53 per cent in British males. This type (or one of the modifications of it) is the one most commonly shown in the modern text books and atlases (Gray, Morris, Cunningham, Poirier and Charpy, Rauber-Kopsch, Spalteholz, Sobotta, and Toldt).

2. The second most frequent arrangement (27.5 per cent) in the American white males was that in which the two longitudinally directed veins (vv. cephalica and basilica) were connected by the M-shaped vv. mediana antibrachii, mediana basilica and mediana cephalica. This type occurred less frequently in the American negro males (12.1 per cent) than in the American white males. In the Japanese it appeared in only 1 per cent of the cases, whereas Berry and Newton observed this type in 16 per cent. Illustrations of this arrangement appear in Quain's Anatomy, in Piersol's Anatomy, in Poirier and Charpy's Anatomy, in Sobotta's Atlas, and in Told's Atlas.

3. In the American negro males the second most frequent arrangement (14.5 per cent) was one in which the two longitudinally directed veins (vv. basilica and cephalica) were without cross connection in the region of the cubital fossa. This type occurred only on the left side in the American white males (3.3 per cent). Okamoto reports a frequency of 14.5 per cent in Japanese, while Berry and Newton found this type to be present in only 0.7 per cent in the British subjects studied by them.

4. Absence of the v. cephalica in the arm (type V) was observed in only 1.6 per cent of all cases (2.4 per cent American negroes and 0.8 per cent American whites). This condition was found in only 0.3 per cent in British and did not occur in Japanese. Sobotta's Atlas seems to be the only atlas or text book containing a plate showing this venous pattern.

5. In about 15 per cent of the cases the v. cephalica had a so-called 'Inselbildung' (reduplication of the veins) in its course (type VI A). This occurred with about the same frequency in both American white and American negro males. In Japanese 27 per cent of the arms showed this condition, but in British males it occurred in only 16 per cent. 'Inselbildung' in the course of the v. basilica was observed in only 2.5 per cent of the American white and American negro males; in the Japanese it occurred in 1 per cent of the arms. It was not reported by Berry and Newton.

6. Termination of the v. mediana antibrachii was most frequently (about 45 per cent) into the v. mediana cubiti in all groups, except on the left side of American negro males. The next most frequent termination of the v. mediana antibrachii was into the v. basilica (about 35 per cent). Termination of the v. mediana antibrachii by dividing into the vv. mediana basilica and mediana cephalica occurred more often in American white males (27.5 per cent) than in American negro males (12.1 per cent); this type appeared in 1, 10, and 13 per cent of the Japanese, Finns, and British, respectively.

7. Presence of an accessory v. mediana cubiti was more frequent in American white males (4.2 per cent) than in American negro males (0.8 per cent). This vein occurred in 8.5 per cent of the Japanese, in 4 per cent of the British, and in 1.2 per cent of the Finnish males and females.

8. In American white, American negro, and Japanese males the right side showed a greater incidence than the left side of the v. cephalica accessoria, 'Inselbildung' of the vv. cephalica, and reduplication of the v. mediana cubiti. No other differences between the two sides were apparent.

9. The American white males differ from the American negro males in that type III was more frequent in the former group, whereas type IV was more frequent in the latter group. The British males resembled the American white males very closely, while the Japanese did not show any marked consistency in similarity to any of the other groups. It should be noted that in the latter group type IV was of the same frequency as in the American negro males.

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THE HISTOLOGY OF THE ANTERIOR PITUITARY OF THE ALBINO RAT IN RELATION TO THE OESTROUS CYCLE [1]

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ONE PLATE (FOUR FIGURES)

Experimental evidence of a definite pituitary-gonadal relationship in mammals began with the work of Evans ('24), who showed that injections of saline extracts of ox anterior pituitary were capable of inhibiting the oestrous cycle in the rat. This suppression, moreover, was found to be associated with striking modifications in the ovaries. The changes consisted essentially in the luteinization of larger follicles through the formation of corpora lutea atretica, resulting in the production of large luteal masses in which were embedded remains of the ova. The functional capacities of corpora lutea formed in this manner were subsequently demonstrated by Parkes ('29) and by Teel ('26).

Smith ('26, '27, '30) has shown that hypophysectomy in the rat promptly causes cessation of the oestrous cycle followed by genital atrophy, and reported further that implants of hypophyseal tissue corrected these changes. In 1927, Zondek and Ascheim as well as Smith and Engle independently reported the results of experiments in which it was shown that implants of fresh anterior lobe into immature rats and mice resulted in a precocious sexual development, thereby demonstrating very clearly the functional control exercised by the pars anterior over the gonads and, through them,

[1] Reported before the American Association of Anatomists, Charlottesville, Virginia, April, 1930. (Abstract no. 7, p. 210.)

over the genital system as a whole. The active principle in the fresh material utilized by these workers, differing in its effect from that employed by Evans, gave rise to the conception of at least two substances originating in the anterior pituitary—a growth principle and a maturity principle.

The nature of the sexual rhythm, with its alternate periods of growth and of regression, led Smith and Engle ('27) to suspect, on theoretical grounds, that a rhythmic or periodic secretion of the gonad-stimulating hormone might best account for the cyclic nature of the female reproductive system. It has been suggested by other investigators (e.g., Evans and Simpson, '28), that the presence of two hormonal principles might conceivably be associated with the occurrence of two well-known cell types, notably the basophils and the eosinophils, particularly on the basis of clinical evidence regarding the coincidence of eosinophilous adenomata, or in some instances of a pronounced basophilia, with certain types of growth or metabolic imbalance. It appears possible that, should a rhythmicity exist in the normal animal, its presence might be reflected in the histological structure of the pars anterior and that examination of tissue obtained from animals in different phases of the oestrous cycle would conceivably yield circumstantial evidence in support of such a hypothesis. It was thought advisable to make such a study, using the rat as material, and the present report deals with results of observations derived from a careful study of the rat pituitary.

MATERIAL AND METHODS

A large number of healthy virgin females, the regularity of whose cycles had been determined by the vaginal-smear method over a three-month period, were isolated and divided into two groups, litter mates appearing in each group. The animals in group A were sacrificed during the late oestrous phase, those in the remaining group B were killed during the late dioestrous interval. In addition to these, a number of pituitaries were obtained at random from the stock colony, together with a notation as to the cyclic condition of the respective donors.

As a result of another investigation being carried on by one of the authors (H. O. H.), it was found possible to obtain glands from animals running a continuous oestrus following subtotal oophorectomy or following treatment of the ovaries with phenol. Three incompletely spayed animals comprised group C and three phenol-treated females formed group D. In addition, several animals in the colony proved to be running a spontaneous continuous oestrus and were utilized together, making up group E.

The pituitary from each animal in the various groups was removed in toto following decapitation. The glands from the rats in groups A and B were fixed in Helly's fluid; those from animals in the remaining groups were fixed in Zenker-formol-osmic (Guthrie). The tissues were then embedded in paraffin and frontal, serial sections, 5 μ in thickness, were prepared. The sections were stained in Delafield's haematoxylin and counterstained with alcoholic eosin, and finally mounted in neutral gum damar in xylol.

HISTOLOGICAL OBSERVATIONS

Group A. Pituitaries of late oestrus

The pars anterior of the glands from eight animals in this group show the presence of many basophiles with sparsely scattered, poorly staining eosinophiles (fig. 2). Occasionally a large well-formed eosinophile may be found (fig. 2b). The basophiles contain a compact nucleus surrounded by a goodly amount of light blue, clear cytoplasm. The eosinophiles scattered throughout the pars anterior contain a small reticulated nucleus surrounded by a meager amount of pink-tinted, finely granular cytoplasm. Occasional chromophobic and neutrophilic cells can be found in some of the nests. In general, the glands appear to be compact and comparatively poorly vascularized.

Group B. Pituitaries of late dioestrus

Pars-anterior material from the animals in group B show the presence of many comparatively large eosinophiles inter-

spersed with occasional, small, poorly staining basophiles. The eosinophiles contain a well-formed vesicular nucleus embedded in a mass of deeply red staining, finely granular cytoplasm. The cytoplasm forms a fairly wide rim about the nucleus (fig. 1). The basophiles, occurring singly or in pairs in the various nests of cells, contain a compact nucleus surrounded by a narrow margin of light blue cytoplasm (fig. 1a). Occasional neutrophiles, but no chromophobe cells, can be found in some of the fields examined. In general, the glands appear to be less compact than those obtained from group-A animals and are comparatively more vascular.

Groups C and D. Pituitaries of phenol-treated and from partially spayed animals

Anterior pituitaries of these animals show the presence of groups and columns of cells. These groups and columns appear made up for the most part by large cells containing vesicular nuclei surrounded by a goodly amount of light blue tinted cytoplasm. The cytoplasm of these cells appears clear and homogeneous (fig. 3). An occasional small cell containing a compact nucleus in a sparse amount of pink-tinged cytoplasm may be found in the main cell nests. The sinusoids surrounding the cell groups contain many blood elements. In general, the pars anterior of these glands presents a histological picture in which the basophilic elements are dominant. The glands appear to contain more cellular material than those in either group A or B, and while fewer sinusoids are present per square area, yet those small vessels present appear more engorged (fig. 3).

Group E. Pituitaries of spontaneous continuous oestrus

In general, the histological picture presented by these glands is the same as that described above for the pars-anterior material from phenol-treated and from subtotally spayed animals. The essential, most apparent difference seems to be in the nature of the cytoplasm of the large basophilic cells

so dominant in this type of gland. The cytoplasm in these cells (fig. 4) is finely granular and not smooth and homogeneous. The granules, as well as the cytoplasm, exhibit a light blue tinge.

DISCUSSION

Evidence pointing toward alterations in hormonal content or release, cyclic and otherwise, has been accumulating recently—much of it, indeed, since the present work was begun. Much of the data is physiological, although scattered histological observations may be found in the literature dealing with the anterior pituitary.

Engle ('29) found the ovarian response of immature rats to castrate pituitaries significantly greater than their reaction to normal glands, i.e., the castrate pituitary was found to contain a greater hormonal content—a fact which he attributed "either to a greater rate of secretion within the cell after gonadectomy, or to a storage of the hormone after its elaboration within the cells of the gland." Engle's findings were subsequently confirmed by Evans and Simpson '29 a). The histological observation of Addison ('17) that the basophilic elements increase in size and number after castration appears significant in this connection. Evans and Simpson ('29 b) found no significant difference between pregnant and non-pregnant glands with respect to their physiological effect, whereas Bacon ('30) reported the pregnant pituitary (beef) to be relatively poorer in hormonal content. Philipp ('30) reported somewhat similar results with human material, that is, the pregnant pituitary proved considerably less efficacious than the non-prenant in inducing precocious maturity. Smith and Dortzbach ('29) reported that the foetal pig pituitary, as judged by efficacy of implants, gives evidence of the presence of the growth principle at a considerably earlier stage than that at which the gonad-stimulating principle appears; the latter was not detectable before the 17-to-20-cm. stage. Burch and Cunningham ('30) found that pituitaries from oestrin-treated castrate females were more efficacious than were glands from untreated castrates. On the

other hand, Leonard, Meyer, and Hisaw ('30) reported that injection of oestrin for some time (twenty to twenty-seven days) lowered the ovarian-stimulating capacity of the anterior lobe. Mason and Wolfe ('30) reported an increase in ovarian-stimulating capacity as a result of a vitamin-A or a vitamin-E deficient diet. Evans and Simpson ('30) demonstrated a lowered capacity in pituitaries from animals fed a diet deficient in antineuritic vitamin-B.

From the foregoing, therefore, it is obvious that alterations, in secretion or in release, may be effected either through the intervention of pregnancy or by experimental means.

So far as we are aware, only two reports have appeared presenting evidence of a variation in hormonal principle during the normal sex cycle. Smith and Engle ('29) found that a variation occurs in the ovarian-stimulating capacity of the anterior lobe directly related to the phase of the non-pregnant cycle (guinea-pig), glands from animals in oestrus showing relatively little physiological efficiency, in contradistinction to those from animals in the interval. Wolfe ('31) reported a cyclic variation in the sow pituitary with respect to its capacity to induce ovulation in the rabbit; its efficacy being greatest during the interval, considerably less during oestrus, and least of all during pregnancy.

Among the papers pointing out histological conditions in the anterior pituitary which might possibly be correlated with certain physiological or pathological states, it is of interest to note that of Bailey and Davidoff ('25). In an effort to determine whether there is an apparent histological characteristic accompanying acromegaly, they reported the presence of a constant eosinophilia. Smith and Smith ('23), using as a basis the fact that the ox anterior pituitary is grossly separated into a central predominantly basophilic area and a cortical eosinophilic region, implanted the separated parts into hypophysectomized tadpoles and found the growth principle largely confined to the outer, eosinophilic portion, whereas the central basophilic core appeared to contain the larger concentration of endocrine-stimulating substance. Evans and

Simpson ('28), who showed a clear-cut antagonism between sex and growth hormones in that animals (rats) injected with both did not respond to the sex principle, believed, on the basis of Bailey and Davidoff's observations, that there are two principles liberated, "in consonance with the well-known occurrence of two distinct granular cell types, the basophils and the eosinophils," and that the former cell type may be responsible for the secretion of the sex principle, the latter of the growth hormone. The belief that the eosinophiles may contribute a growth hormone was also held by Cushing and Davidoff ('27). It is notable, in this connection, that the castration pituitary, characterized by its basophilic condition (Addison) contains considerably more of the sex principle than does the normal gland. Engle ('29) comments upon this fact and states "one is led to suspect that it is the basophilic cell which elaborates the gonad-stimulating factor of the anterior lobe, although definite statement to this effect seems unwarranted at this time."

Subsequent to Smith and Dortzbach's observations on a variation in the foetal pig pituitary correlated with the stage of development, Nelson ('30), from histological examination of a graded series of pig embryos, reported in a preliminary paper "a distinct histological difference which may be related with the physiological one found by Smith and Dortzbach." Nelson, working in this laboratory, found a distinctly basophilic condition present at the 10-cm. stage—the stage at which Smith and Dortzbach found the growth principle present. The 20-cm. stage, on the other hand, was decided eosinophilic—the stage at which the gonad-stimulating principle was readily demonstrable. These observations, as yet not available in a complete paper, while at variance with the more generally accepted idea regarding cell types and secretion (compare Evans and Simpson; Engle; Cushing and Davidoff), nevertheless, indicate that a variation does occur in the general histological picture more or less coincident with the predominant type of secretion.

Histological observations on the pituitary during the sex cycle apparently have been confined to those reported by Rasmussen ('21), who found a basophilic condition in the anterior pituitary of the woodchuck during oestrus—an observation with which the present results on the rat are in accord. Wolfe, Cleveland, Campbell ('32) reported the existence of a cyclic variation in the pars anterior of the dog in relation to the oestrous cycle.

In the present work a cyclic variation in cell types is demonstrated in the rat—a variation which appears to occur pari passu with the two major phases of the sex cycle, oestrus and the dioestrous interval. During oestrus the general picture is a basophilic one; during the interval it is eosinophilic. An interpretation of these conditions at the present time would be hazardous; moreover, the short cycle of the rat does not render it a particularly suitable form for an interpretative study. A detailed study of the glands of forms with longer cycles would be highly desirable, but, to date, this has not been done. Whether the eosinophiles liberate a growth hormone during the interval, or whether the basophiles liberate a sex principle during oestrus would be difficult to postulate on the basis of the present work. Physiologically, it would appear necessary that a certain interval elapse before the principle secreted by any cell could give evidence of its action. Injections of a gonad-stimulating substance, for example, require at the least several hours before their effect becomes apparent, more usually a day or two. In view of the short cycle in the rat, therefore, it is not impossible that the eosinophiles of the interval liberate the gonad-stimulating principle which brings about oestrus and ovulation a day or two later, and that the basophiles actually liberate the growth hormone. Furthermore, the dangers inherent in attempting to homologize clinical evidence with experimental results render any attempts at arguing by analogy unsatisfactory.

One fact appears obvious from the present work, namely, that the pituitary is a highly sensitive, readily reactive gland,

as evidenced by the fact that, in the rat with its extremely short cycle of four to five days, the pars anterior undergoes definite cyclic variations in its histological structure. That there is a variation is demonstrable; the functional significance of the histological rhythm remains obscure.

SUMMARY

1. The anterior pituitary of the female albion rat undergoes a definite cyclic rhythm in predominant cell type present, as revealed histologically. This rhythm can be correlated with the phase of the sex cycle present when the gland was obtained. During oestrus the anterior lobe is found to be predominantly basophilic; during the dioestrus interval it presents an eosinophilic picture.

2. In instances of experimentally induced or of spontaneous continuous oestrus the pars anterior is characterized by a pronounced basophilia, essentially similar to that occurring during normal oestrus in the cyclic animal.

3. Owing to the shortness of the rat cycle and in view of our extremely scant knowledge of histological and physiological correlation of the anterior pituitary, an attempt at interpretation of the respective functions of the two major cell types seems unwarranted at the present time.

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PLATE 1

EXPLANATION OF FIGURES

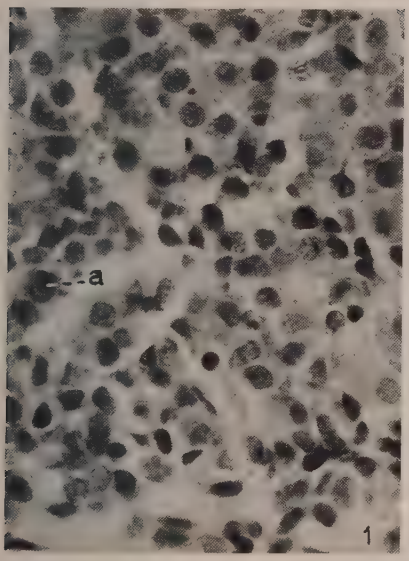
All four figures in this plate are photomicrographs taken at the same magnification (oil immersion, × 950) with the same optical setup and are printed on the same grade contrast sensitized paper.

1 Section through the pars anterior of an adult albino rat sacrificed at the late dioestrous phase of the cycle. Notice the numerous large cells containing a vesicular nucleus. *a*) Basophile cell with compact nucleus.

2 Section through anterior pituitary from animal sacrificed during the late oestrous phase of the cycle. Note the compactness of the cells as compared with figure 1. and the numerous cells with compact nuclei, the basophiles. *b*) Large well-formed eosinophile.

3 Section through the anterior pituitary of an animal which had been subtotally oophorectomized. Compare with figures 1 and 2. Notice that the cells are larger and the field is made up of more cellular material than the fields shown in the first two photographs. A mass of blood elements may be seen in the small vessel coursing through the field.

4 Section through the pars anterior of a pituitary continuous oestrus. Compare this with figure 3 and notice the granular nature of the cytoplasm of most of the cells.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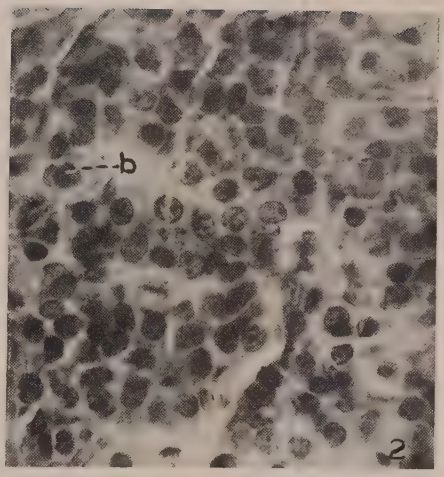
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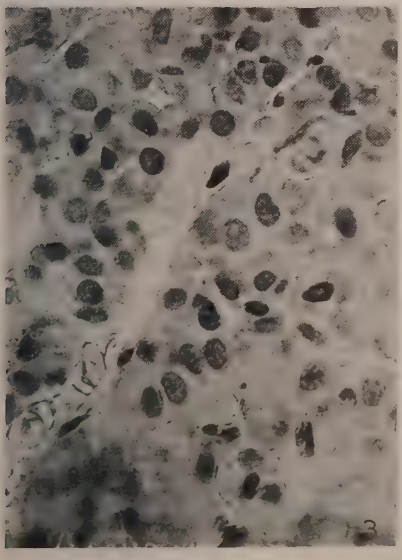
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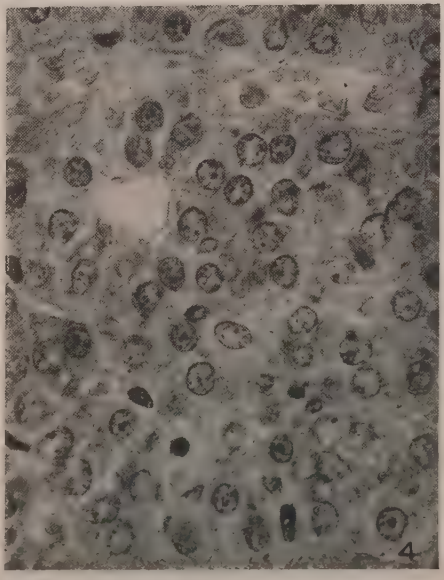
4

THE GALL BLADDER AND THE EXTRAHEPATIC BILIARY PASSAGES IN LATE EMBRYONIC AND EARLY FETAL LIFE

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FOUR PLATES (SEVEN FIGURES)

The early stages of the development of the biliary system have frequently been investigated, but little information is available on the human gall bladder and the biliary passages in late embryonic and early fetal life(1,2). Adequate material for such studies is, of course, difficult to obtain. It was our good fortune to secure some preparations suitable to furnish information on some points of particular interest regarding the structural evolution of the gall bladder and the extrahepatic biliary passages. The data obtained are presented in this preliminary report.

MATERIAL

There were at our disposal: 1) Serial sections of the entire biliary system of two embryos, 25 and 40 mm. long, and, 2) serial sections of eight gall bladders of embryos and fetuses ranging from 80 to 370 mm. in length. Most of the sections were stained with hematoxylin eosin, and some by the method of van Gieson.

A. The gall bladder

The endodermal portion of the gall bladder in both the 25- and 40-mm. embryos is represented by a single layer of columnar epithelial cells which line the lumen, the mesodermal portion comprising the rest of the wall (fig. 1).

The mucosal surface is smooth and devoid of elevations and indentations. The cells of the endodermal epithelial lining appear uniform in size and staining reaction. Only here and there are seen cells in a state of mitotic division. The cell nuclei are large and contain granules which are deeply stained and evenly distributed. The location of the nuclei is, with slight variations, in the midportion of the cell. The cytoplasm is stained lightly except for a narrow strip of the free margin. However, the intensely stained border of the gall-bladder epithelium is not a true cuticula. This becomes evident by a comparison with the cuticular margin of the intestinal epithelium. At this stage of development the mesodermal portion of the gall bladder shows only a slight indication of differentiation. Although the tunica muscularis cannot be recognized as such, a zona muscularis may be tentatively separated from the zona subepithelialis and the zona perimuscularis. In the zona subepithelialis, which is a narrow band encircling the epithelial lining, the cell nuclei are round and very numerous. In the zona muscularis, from which the muscular coat is going to develop, the nuclei are oval and less numerous. Young muscle cells and capillaries in process of formation may be recognized in this layer. Up to the periphery of this zone, the gall-bladder wall is alike on both its peritoneal and liver surfaces. The next zone, the zona perimuscularis, is broader toward the liver, attaining a width almost equal to that of the zona subepithelialis and zona muscularis combined. Here the cell nuclei are round, sparse, and evenly distributed. An occasional vessel is seen in process of formation. Along the line of attachment to the liver a collection of round nuclei is noted. On the peritoneal aspect of the viscus the perimuscular layer is a barely perceptible, narrow zone between the muscularis and the peritoneal serosa. A comparison with the intestine reveals the remarkable fact that the differentiation here has progressed much farther than in the gall bladder. The mucosa of the intestinal wall is thrown into folds, the lamina propria mucosae is distinct, a zona muscularis mucosae is indicated,

and the tunica muscularis contains muscle cells already arranged into an inner circular and an outer longitudinal layer.

The gall bladder of both the 80- and 95-mm. embryos shows distinctly the differentiation into layers (fig. 2). In the mucosa the formation of folds has begun; alveolar indentations and corresponding rounded elevations give a pattern to its surface. This is particularly marked in the neck where the lumen is minute and is composed of from two to six communicating epithelial-lined indentations. The tunica propria mucosae is a thin layer of connective tissue with numerous blood capillaries. On this layer rests the epithelial lining. The subepithelial layer is surrounded by the tunica muscularis which is a well-defined layer composed of smooth-muscle cells embedded in a connective-tissue stroma. This tunic is rather complete, there being no interruption of its continuity by penetrating epithelial indentations of the mucosa. The perimuscular layer is slightly denser in its portion adjacent to the muscular coat. This denser zone contains the coarse ramifications (plexus perimuscularis) of the large blood vessels. The width of the perimuscular layer almost equals that of the mucosa and muscularis combined. A structural difference in the portion attached to the liver and that covered by peritoneum is not apparent.

The gall bladders of the 115-, 120-, and 130-mm. fetuses show further progression in the differentiation of their structures (fig. 3). The surface pattern of the mucosa is rich in elevations and indentations. In the neck an occasional acinar outpouching of the lumen appears external to the muscular coat. The cytoplasm of the lining cells of these outpouchings are stained lightly and their nuclei placed at the base of the cells. Evidently they represent glands which, at this site, make their first appearance in this period of development. Another structure, the 'true Luschka ducts,' remnants of the liver primordium, are first seen in sections of the 130-mm. fetus. They appear as groups of deeply stained cuboidal cells situated in the periphery of the perimuscular layer on both the liver and the peritoneal sides of the viscus.

The gall bladders of the 200-, 280-, and 370-mm. fetuses are miniatures of that of the newborn (figs. 4 to 6). Rapid advance in the development of the mucosal pattern is a prominent feature of this stage. The folds of Heister and the curves of the neck now assume definite forms (fig. 4). The active proliferation of the lining epithelium is rather conspicuous in sections from the gall bladder of a 370-mm. fetus (fig. 5). The arrangement of the muscular tunic into an incomplete inner longitudinal and outer rather oblique layer is seen. The distribution of the vascular supply is strikingly brought out in the injected specimen of a 280-mm. fetus (fig. 6). The coarse ramifications (plexus perimuscularis) of the large vessels are located in the dense zone of the perimuscular layer, in that adjacent to the muscular coat. Surrounding the muscular coat are six to twelve small arteries, placed equidistant from one another, with their concomitant veins. Branches of these vessels communicate through the muscular layer with the rich capillary bed (plexus subepithelialis) distributed beneath the lining epithelium.

B. The hepatocystic and the choledochoduodenal junctions

The endodermal portion of the extrahepatic biliary ducts in both the 25- and 40-mm. embryos is represented by a tube lined with a single row of columnar epithelial cells. The mesodermal portion is wholly undifferentiated. The lumen of the cystic duct merges gradually with that of the common hepatic duct (fig. 7). The course of the ductus choledochus can be easily followed in serial sections and its gradual penetration together with the pancreatic duct on the dorsomedial side of the duodenum observed (fig. 1). At this stage of development the differentiation of muscular tissue in the gall bladder has not occurred, and, as just mentioned, the mesodermal portion of the ductus choledochus is wholly undifferentiated. The duodenal musculature, on the other hand, has already a quite well-developed inner circular and outer longitudinal layer. Thus, it seems reasonable to assume that any muscle tissue present at the choledochoduodenal junction at this stage of development would be of duodenal origin.

In the 200-mm. fetus, the ductus choledochus is quite well formed (fig. 4). Its lumen is lined by a single row of high columnar epithelial cells. There are numerous outpouchings of the mucosa. The deeper portions of some of these outpouchings are lined by low columnar cells which have a lightly staining cytoplasm and a nucleus close to the base. The mesodermal portion of the duct is composed of young connective tissue.

SUMMARY

The evolution of the gall bladder was followed in ten human embryos and fetuses, ranging from 25 to 370 mm.; and the extrahepatic biliary passages in serial sections of two of the embryos and one of the fetuses.

In the 25- and 40-mm. embryos the endodermal portion of the gall bladder is represented by a single layer of columnar epithelial cells which line the lumen. In the mesodermal portion, comprising the rest of the wall, the zona muscularis is barely indicated, while the tunica muscularis of the intestine is already well defined. In the 80- and 95-mm. embryos the wall of the gall bladder is differentiated into layers. In the 115-, 120-, and 130-mm. fetuses the surface pattern of the gall-bladder mucosa is rich in elevations and indentations. The glands in the neck appear and the 'true Luschka ducts' are first seen. In the 200-, 280-, and 370-mm. fetuses the gall bladder is a miniature of that of the newborn; the curves of the neck and the folds of Heister appear.

The endodermal portion of the extrahepatic biliary ducts in both the 25- and 40-mm. embryos is represented by a tube, lined with a single row of columnar epithelial cells; the mesodermal portion is wholly undifferentiated. In the 200-mm. fetus the endodermal portion of the ductus choledochus is well formed, the lining epithelium showing numerous outpouchings and glandular structures; the mesodermal portion is composed of young connective tissue.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Gall bladder and duodenum of a 40-mm. human embryo; cross section at the level of the umbilicus. The cells of the endodermal epithelial lining appear uniform in size and staining reaction. The mesodermal portion of the gall bladder shows only a slight indication of differentiation into layers. The passage through the duodenal wall of the ductus choledochus and the ductus pancreaticus is seen. Photomicrograph, × 50.

2 Gall bladder of an 80-mm. human embryo; cross sections from the corpus (*a*) and from the neck (*b*). The alveolar indentations and corresponding round elevations give a pattern to the mucosal surface. This is particularly marked in the neck, where the stellate lumen is composed of several communicating epithelial-lined indentations. The muscular coat is a well-defined layer of smooth-muscle cells embedded in a connective-tissue stroma. Photomicrographs, × 60.

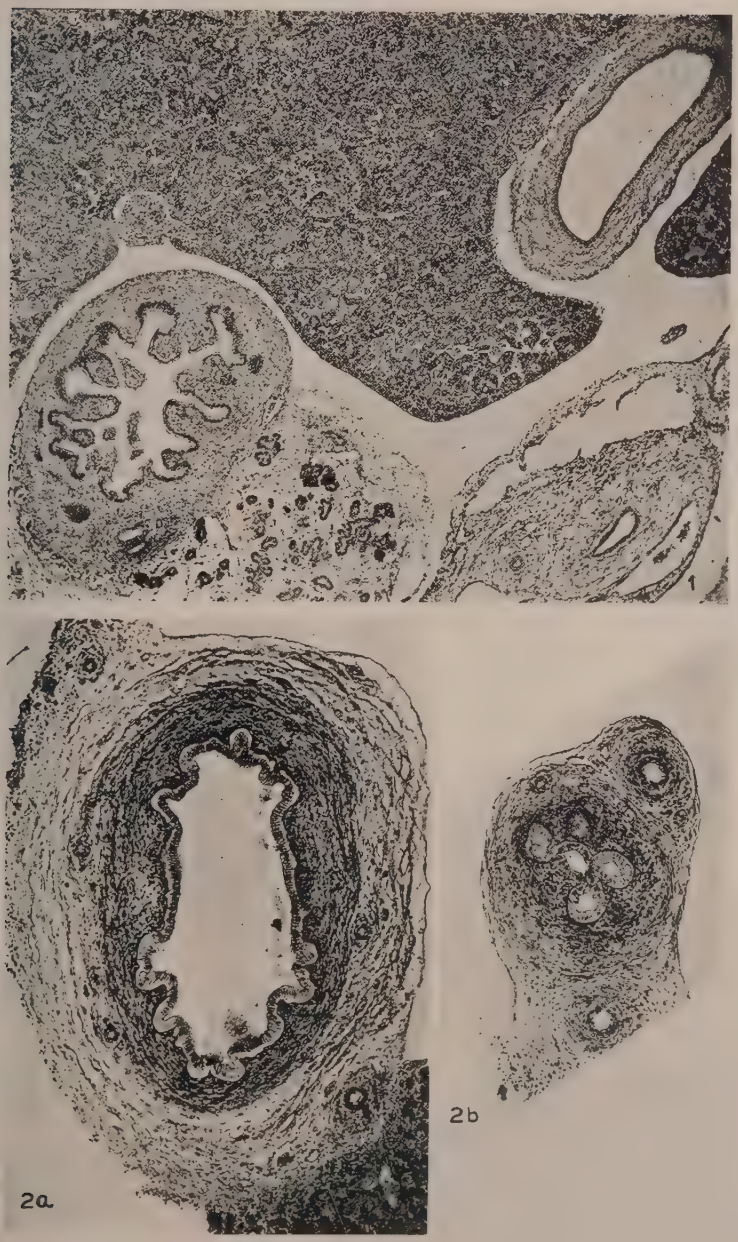
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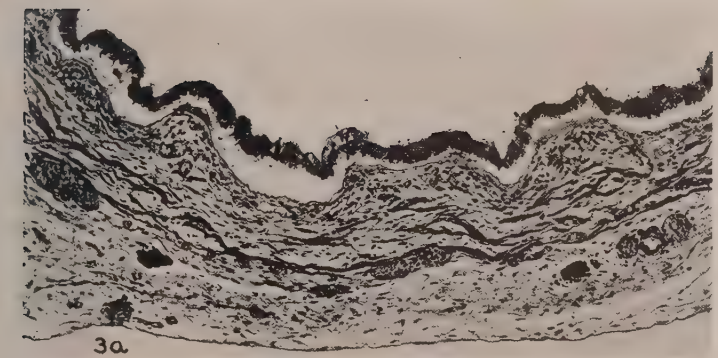
PLATE 2

EXPLANATION OF FIGURE

3 Gall bladder of a 130-mm. fetus; cross sections from the corpus (*a*) and from the neck (*b*). The surface pattern of the mucosa is rich in elevations and indentations. Two groups of deeply stained cuboidal cells situated in the periphery of the perimuscular layer are remnants of the liver primordium. These 'true Luschka ducts' make their first appearance at this stage of development (*a*). Some of the acinar outpouchings of the lumen of the neck appear external to the muscular coat and are lined by low columnar cells stained lightly and having their nuclei in the base of the cells (*b*). Photomicrographs, × 100.



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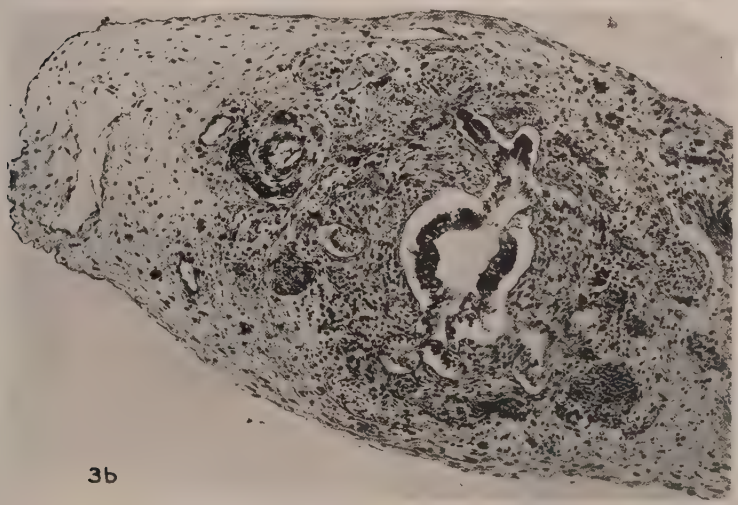

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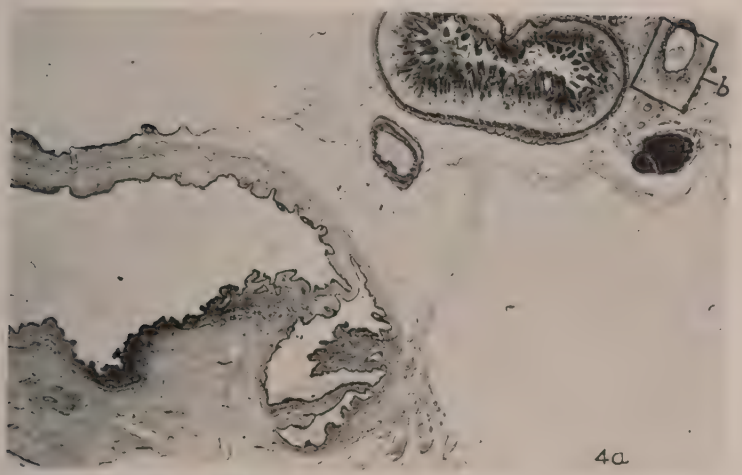
PLATE 3

EXPLANATION OF FIGURE

4 Gall bladder of a 200-mm. fetus in longitudinal section. The folds of Heister and the curves of the neck assume definite forms. The viscus is the miniature of that of the newborn (*a*). The ductus choledochus is quite well formed. The mucosa shows numerous outpouchings. The mesodermal portion of the duct is composed of young connective tissue (*b*). Photomicrographs, (*a*), × 12; (*b*), × 120.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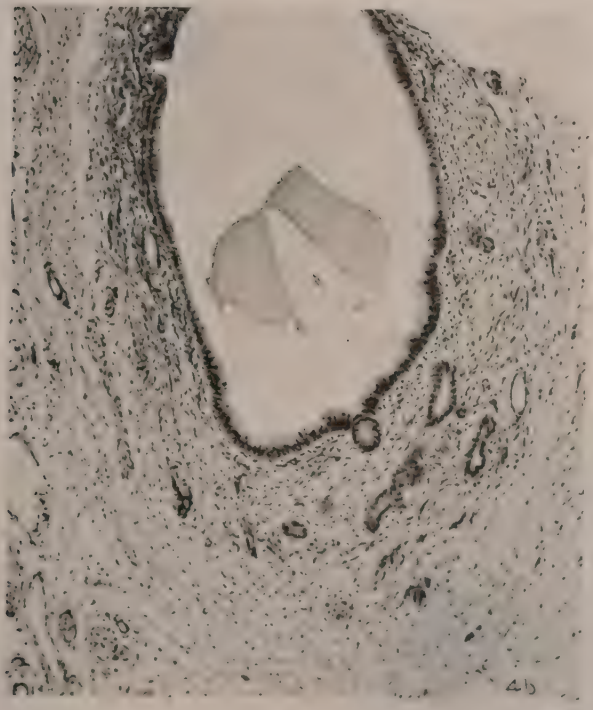
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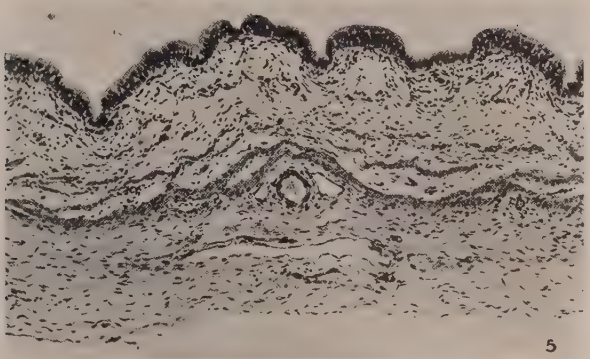
PLATE 4

EXPLANATION OF FIGURES

5 Gall bladder of a 370-mm. fetus in longitudinal section. The active proliferation of the lining epithelium is rather conspicuous. Photomicrograph, × 120.

6 Gall bladder of a 280-mm. fetus in cross section. The coarse ramifications (plexus perimuscularis) of the large vessels are located in the dense zone of the perimuscular layer, in that adjacent to the muscular coat. Branches of these vessels communicate through the muscular layer with the rich capillary bed (plexus subepithelialis) distributed beneath the lining epithelium. Photomicrograph, × 60.

7 The hepatocystic junction of the 40-mm. human embryo. The lumen of the cystic duct merges gradually with that of the common hepatic duct. The endodermal portion of both ducts is represented by a tube lined with a single row of columnar epithelial cells. The mesodermal portion is wholly undifferentiated. Photomicrograph, × 120.



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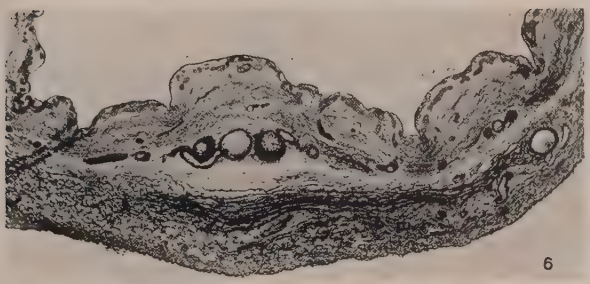

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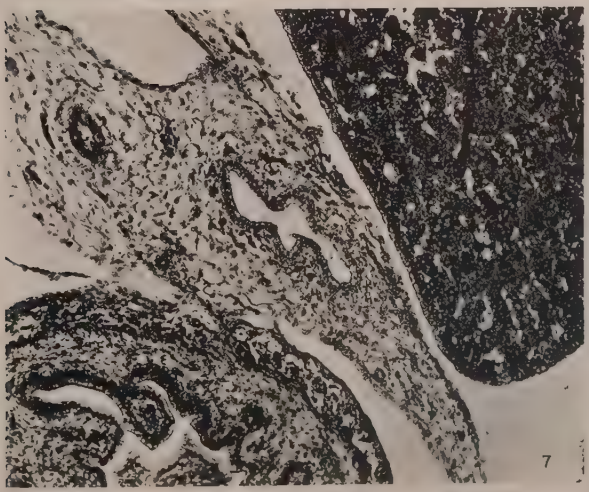


7

THE RESPONSE OF BUFO LARVAE TO DIFFERENT CONCENTRATIONS OF THYROXIN

BENNET M. ALLEN[1]
University of California at Los Angeles[2]

THIRTEEN FIGURES

The well-known effects upon amphibian metamorphosis produced by feeding thyroid preparations and by transplantation of living glandular substance have been clearly established and yet there is need of quantitative studies on this score in order to solve interesting questions, as for instance:

1. The minimum amount of thyroid hormone to produce evidence of metamorphosis.
2. The degree of response to different quantities of thyroid hormone.
3. The different thresholds of stimulation and rate of reaction of different organs.
4. The capacity of the organism to react to different amounts of thyroid stimulus at different stages of development.
5. The age at which the organism shows a capacity to respond to the hormone.

Certain of these questions have been investigated already by Romeis, '23; Kosmin and Resnitschenko, '27; and by Blacher, '28. Romeis, '23, carried on a series of experiments with tadpoles, his method consisting chiefly in immersing them in the test solution for definite periods and at definite

[1] This work was supported by grants from the National Research Council and the University of California.

[2] It is also a pleasure to express my deep appreciation for the pleasant laboratory quarters placed at my disposal through the kindness of the Del Amo Foundation.

intervals, after which they were placed in normal tap water. He found that thyroxin is far more effective than iodothyrin, iodoglobulin, and diiodotyrosine. The extent and character of the changes produced by thyroxin varies with the concentration of the dose applied, the number of applications, and the age of the animal. There is also the factor of individual susceptibility. Romeis produced definite acceleration in development by concentrations as low as 1 to 1,000,000,000 if tadpoles were kept in this solution during the entire period of their larval life. The limbs were affected in very weak solutions when the larval characters remained unchanged. Kosmin and Resnitschenko studied the effect of thyroxin upon the metamorphosis of the larvae of Rana arvalis in connection with the presence or absence of calcium and potassium salts. This question does not concern us here, but their work is of interest to us because it involved a study of the influence of different concentrations of thyroxin upon the development of Rana arvalis larvae. These investigators immersed lots of ten each of carefully assorted tadpoles of 15 mm. length in thyroxin solutions of 1 to 5,000,000; 1 to 10,000,000; 1 to 20,000,000; and 1 to 40,000,000 in which they were kept for nine days. Observations upon the single point of total length showed it to be reduced in proportion to the concentration of thyroxin in the solution. Another series served to show the influence of a standard thyroxin solution of 1 to 20,000,000 upon sets of tadpoles of different degrees of development. They concluded that the rapidity of response increases with the age of the tadpoles—that the nearer the tissues are to the time of metamorphosis the more readily do they respond to the thyroid stimulus. They stated that tadpoles in the stage of external gills do not respond in the slightest to the thyroid stimulus; but no details are given regarding the evidence on this point. Blacher ('28) studied the threshold of stimulation of different structures of amphibian larvae and the rate of metamorphosis in relation to concentration of the thyroid hormone. In these studies, most significant in relation to our present work, he used thyroidin—the powdered

gland substance. This was made up as an emulsion of different concentrations in tap-water to which a small amount of sodium bicarbonate had been added in order to facilitate the solution of the hormone into the water, concentrations of 1:500,000, 1:250,000, 1:100,000, 1:50,000, 1:20,000, 1:10,000, and 1:5,000 being used. It is seen that the highest concentration is one hundred times the lowest. Three days after the beginning of the experiment all the tadpoles were killed and determinations were made of trunk length, tail length, intestine length, fore limb eruption, and the loss of the horny jaws. Observations were also made upon the assumption of a green color by the gall bladder. In the case of each of these characters Blacher noted the lowest concentration of the thyroid suspension that produced visible effects in the uniform period of three days during which the experiment ran. The form used in this experiment was Rana ridibunda in Blacher's 'second stage' of development when the digits of the hind foot are evident and the limb shows angles at the joints. He found that the intestine had the lowest threshold of response, showing definite shortening in a solution of 0.002 per cent within the three days of the experiment. Next in order came the tail (0.004 per cent), trunk (0.01 per cent), loss of horny jaws (0.02 per cent), eruption of fore limbs (0.05 per cent). It does not seem that these threshold differences are due to accessibility of the part to the action of the solution, but to differences in the capacity of the part to respond. As evidence he cites the slight response of the fore limb anlagen in spite of the fact that Helff had shown their dependence upon the degeneration of the gills—structures in the highest degree accessible to the thyroid hormone as administered under the conditions of this experiment.

One interesting feature is found in the fact that certain concentrations cause maximum effects, and concentrations beyond this do not produce greater effects and may even bring about less development than the optimum. In other words, the curve of growth shows a descent at the higher concentrations. This was found to be true in the case of each of the structures studied.

LINES OF INVESTIGATION

It is the purpose of this article to add to our knowledge of this subject by the following lines of procedure:

1. Application of the thyroid stimulus to tadpoles of stages before the beginning of metamorphosis.
2. Exposure of experimental lots to thyroid hormone for different periods of time.
3. Use of thyroxine, thus giving greater accuracy to application of stimulating agent and avoiding the possibility of nutritinoal influences.
4. Avoidance of nutritional differences due to changes of the alimentary tract induced by maintaining the tadpoles without food during the course of the experiment.

It is believed that we have by these means secured a connected record of the length of exposure necessary to cause different organs to respond to different concentrations of the hormone.

MATERIALS AND METHODS

Tadpoles used in this experiment were obtained in two large, bricked-in pools adjoining the laboratory. They were available in enormous numbers, making possible rigid selection of material. Bufo halophilus tadpoles are relatively small and have strong tendencies to metamorphosis by the time the hind limbs show clear differentiation into parts.

Before the experiment was begun the finger bowls in which the tadpoles were to be reared were polished with scrupulous care to remove any deposits from previous experiments. In order to avoid unconscious selection of specimens, the tadpoles were first sorted over to eliminate any specimens either larger or smaller than the stage selected. The finger bowls were then placed on a table and the tadpoles distributed among them one at a time, adding one to each dish in turn until there were ten in each. Three finger bowls were devoted to each lot representing a given dilution of thyroxin for a given time interval of exposure. The assignment of each finger bowl of tadpoles to its place in the experiment was

made by chance through the familiar device of drawing straws.

The pure thyroxin of Squibb & Co. was used, being carefully weighed, and all precautions being observed to insure accuracy in making up the solution. After dissolving the thyroxin in a few drops of KOH solution it was combined in tap-water. The stock solution was kept only through the period of the experiment. Every two days the tadpoles were placed in 250 cc. of a fresh solution in a clean finger bowl. At chosen intervals of time, different lots were preserved in 5 per cent formaldehyde solutions.

The alimentary tract was pulled loose at the cloaca and at the cranial end of the oesophagus, the mesentery being broken at points where the intestine is thrown into coils and the liver and pancreas dissected off. It was then extended by being placed in a groove made by laying one microscope slide lengthwise over another in such a position that its edge lies a slight distance behind that of the other. A small amount of moisture affords sufficient surface tension to hold the alimentary tract in position. It must be remembered that the tadpoles were not fed during the course of the experiment, hence the initial shortening of the alimentary tract of the controls during the first few days in each experiment. After this, the curve flattens out and shows far less shrinkage in length. We have here a process of emptying of the intestine that is so heavily distended with coarse food while the tadpoles live in their normal environment.

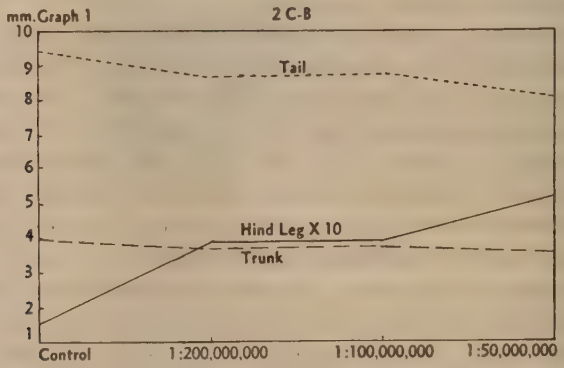
The other measurements were made with dividers and ruler except in cases where they were below 5 mm., when they were taken by means of micrometer readings. In measuring the bent hind limbs, the dividers were applied to each segment and joined together on a straight line from which the total measurement was made.

Four series of experiments were carried out as described in the following pages.

Experiment 2c

In experiment 2c, tadpoles from the same egg string were placed in thyroxin solutions of 1:2,000,000,000, 1:1,000,000,000, and 1:50,000,000 when in the tail bud stage and killed at the end of eighteen days. Measurements of the hind limbs, total length and trunk length were made. Of these the hind limb measurements were the most significant. See graph 1.

We may first point out that the hind limb buds of the controls had barely made their appearance at the end of the



Graph 1 Experiment 2C, series B. Bufo halophilus placed in solution at tail bud stage—total length, 3.37 mm.; tail length, 0.45 mm. Remained in solution for eighteen days. Curves show influence in length of tail, hind legs, and trunk produced by different concentrations of thyroxin acting for the duration of the experiment. Averages of lots of ten each.

experiment. In thyroidectomized and hypophysectomized tadpoles of this species the hind limbs ultimately reach an average length of about 20 to 25 mm. and show clear differentiation into parts before ceasing to develop. Reference to graph 1 will show that the average hind limb length in the controls is 3.9 per cent that of the trunk length, while the hind limbs of thyroidectomized and hypophysectomized tadpoles have a length of 13.6 per cent and 14.0 per cent, respectively, that of the trunk length. In these very young tadpoles of graph 1 we see that the thyroxin treatment had produced a

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ratio of hind leg length to trunk length equal to this only in the case of the most concentrated solution 1:50,000,000. The weaker solutions have induced a hind leg growth in ratio markedly less than this. The point that concerns us, however, is that thyroxin does exert a growth influence in these very young stages. It is significant, however, that this slight influence is the result of eighteen days exposure to these relatively strong solutions. In comparison with the succeeding data it is seen that the response in these early stages is far less than in later stages.

In these young stages, the tail and trunk are shortened to a slight extent while the hind limbs show a great development beyond that of the controls. This is proportional to the concentration of the thyroxin solution, the difference between the 1:50,000,000 and the two less concentrated solutions being very marked. The same cannot be said regarding the 1:100,000,000 and the 1:200,000,000 solutions because the difference in average hind limb length is very slight, but in the expected direction. This is chiefly due to the presence of three unusually vigorous specimens in the 1:200,000,000 series, a complicating feature that can be observed in any mass of eggs. Carefully selected control tadpoles of this group killed in the tail-bud stage showed clear differences in size that would naturally become accentuated as development proceeds.

The thyroxin concentration of 1:200,000,000 is about the weakest capable of producing an effect and it is significant that it produces appreciable effects when applied to these very young stages. We wish to express indebtedness to one of our students, Mr. John Benjamin, for determining this threshold concentration of thyroxin. His unpublished experiments demonstrate the potency of this concentration in experiments upon various stages of several different species of amphibians.

Series 2A

In series 2A, 360 tadpoles were used in twelve lots of thirty each, representing a group killed at the beginning of the

experiment, a control group killed at the termination and lots placed in thyroxin solutions of the following concentrations:

1: 500,000,000	1: 10,000,000
1: 300,000,000	1: 5,000,000
1: 200,000,000	1: 1,000,000
1: 100,000,000	1: 500,000
1: 50,000,000	1: 200,000

The highest concentration is 2500 times greater than the most dilute, but only 1000 times more concentrated than that which our experiments show to be the greatest effective dilution—1: 200,000,000.

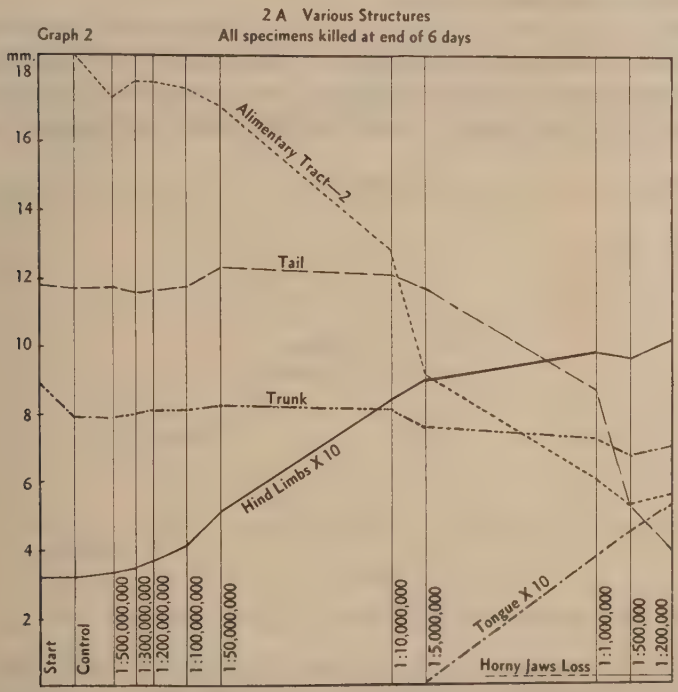
Graph 2 shows the curves based upon the averages of the measurements in this series. In making out this graph it was found convenient to divide by two the data representing the length of the alimentary tract and to multiply by ten the data indicating the length of the hind legs and length of the tongue. In this way, the curves representing these different features could all be brought within the limits of a single graph sheet. The ordinates represent length in millimeters of the given structures and the abscissae represent the concentrations of thyroxin used. The positions of these points are arranged in such a way that an interval of 10 mm. represents a doubling of the concentration of the solution.

It is quite obvious that there is no intention of making these curves comparable with one another in any quantitative sense, although these liberties that we have taken with them make it easier to compare them.

The alimentary tract, trunk and tail shorten, while the hind limbs and tongue lengthen. It is clear that these changes strike the alimentary tract and hind limbs in weaker solutions than those that affect the trunk and tail. We can thus say that they have a lower threshold of stimulation within the six-day time limit employed.

Of these measurements, the tongue offers some difficulties, especially at the beginning of its development, so that we merely indicate its first stages of growth by means of a + mark.

It is difficult to make an accurate determination of the loss of the horny jaws because the early stages of this process show only partial loss.



Graph 2 Experiment 2A. Bufo halophilus placed in various indicated concentrations of thyroxin. Dimensions at starting point of experiment indicated in graph. Abscissae so spaced that 7 mm. equals doubling of concentration. Averages of lots of thirty each.

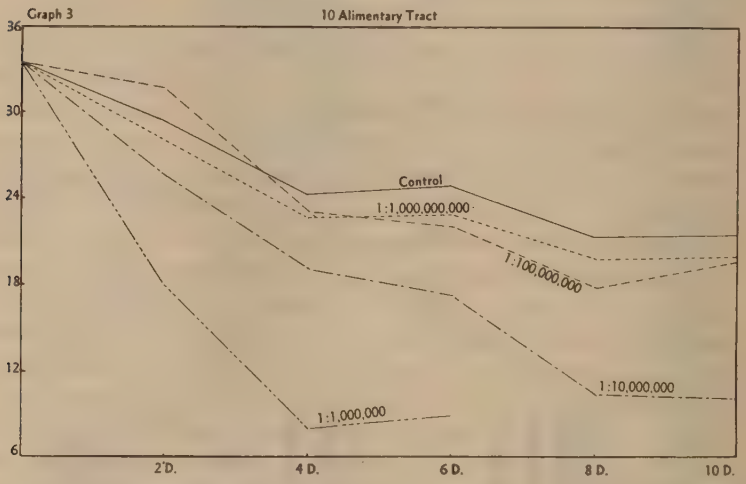
Experiment 10

Experiment 10 includes twenty-four lots of from eight to ten specimens each. There are certain inequalities in the results partly due to the small number of specimens in each lot, but more especially due to the fact that the work was carried on late in the season when the tadpoles had begun to become scarce and it was no longer possible to be so dis-

criminating in the selection of specimens as in the other series.

The graphs give the data in summary upon which the following statements are based.

Alimentary tract. Keeping in mind the general remarks regarding the initial shrinkage of the alimentary tract due to inanition during the experiment, we see that its true shortening does not begin to come into evidence until about the

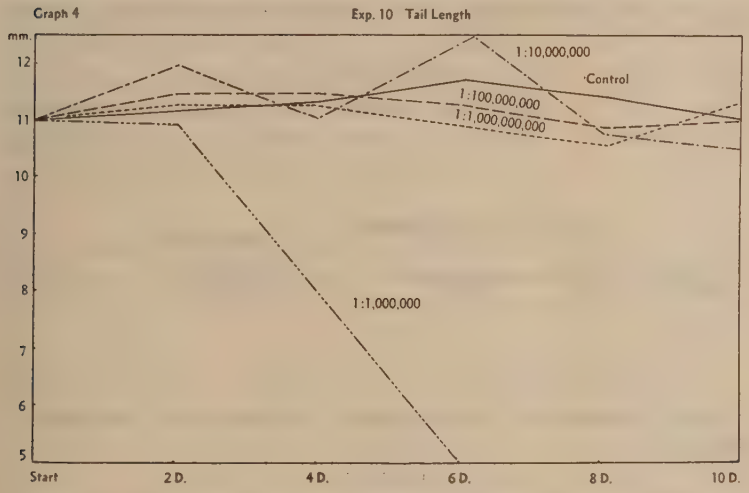


Graph 3 Experiment 10. Bufo halophilus. Alimentary tract length. Lots of ten each placed in various concentrations of thyroxin for various periods of time indicated in days, *D.* Measurements in millimeters. Each curve indicates the influence of the indicated concentration of thyroxin acting through the stated periods of time.

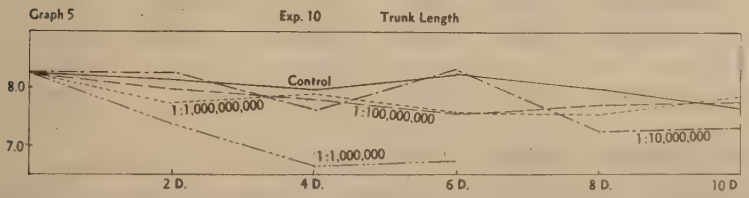
sixth day in solutions of 1:100,000,000. This seems fairly clear from the data, but its validity will be discussed in another place. The solution of 1:10,000,000 exerts a far more powerful influence and the response is seen to come very quickly after the beginning of the experiment.

The tail length does not respond in any convincing degree to the 1:10,000,000 solution during the entire course of the ten days of this experiment; but does respond sharply to the solution of 1:1,000,000 on the fourth day (graph 4).

The measurements of trunk length, graph 5, tell very nearly the same story with some evidence of decrease in length under the influence of the 1: 10,000,000 thyroxin solution on the



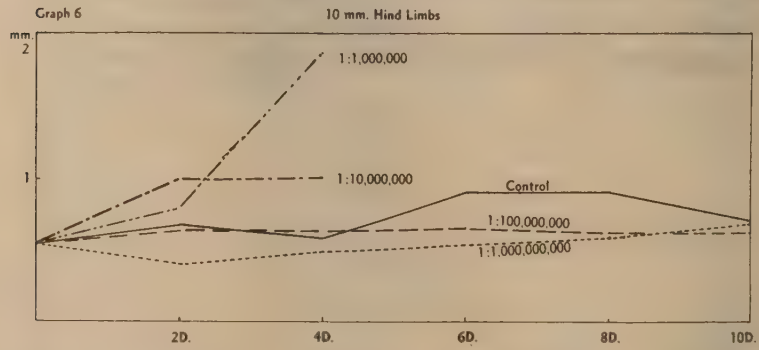
Graph 4 Experiment 10. Bufo halophilus. Tail length. See legend of graph 3.



Graph 5 Experiment 10. Bufo halophilus. Trunk length. See legend of graph 3.

eighth day and a definite decrease caused by the 1: 1,000,000 solution beginning with the second day. This is in line with the reduction of the alimentary tract and may be in part due to it although it is probably more fundamental, being caused, no doubt, by a shortening of the vertebral column.

The curves showing the growth of the hind limbs (graph 6) show a response to thyroxin in solutions of 1: 10,000,000 and 1: 1,000,000 at the end of two days, and the solution of 1: 1,000,000 produces a marked growth at the end of four days. It is clear that the lots in the greater concentrations of this



Graph 6 Experiment 10. Bufo halophilus. Hind limb length. See legend of graph 3.

GRAPH 7

Experiment 10. Horny jaws

Control	XX 10	XX 9	XX 9	XX 8	XX 8
1: 1,000,000,000	XX 10	XX 10	XX 10	XX 10	XX 10
1: 100,000,000	XX 10	XX 10	XX 10	XX 10	OX 9 OO 1
1: 10,000,000	XX 10	XX 10	OX 9 OO 1	OX 9	OX 2 OO 8
1: 1,000,000	XX 10 2 D.	OO 10 4 D.	OO 10 6 D.	8 D.	10 D.

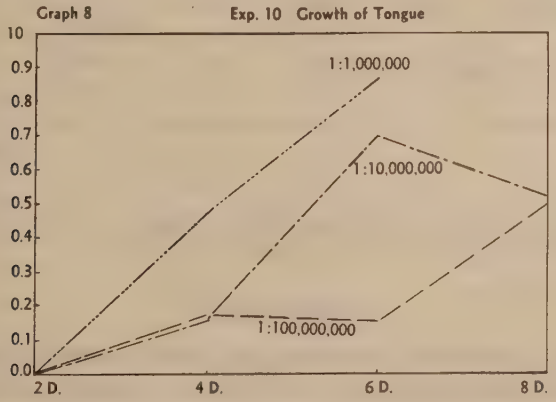
Graph 7 Experiment 10. Bufo halophilus. Horny jaws. XX indicates presence of horny jaws. OX indicates their partial presence. OO indicates their absence.

series have reacted more strongly than those in the weaker concentrations. As stated above, we had a small supply from which to select the tadpoles of this experiment and this resulted in some inequalities in them.

The loss of the horny jaws is indicated in graph 7. With a thyroxin solution of 1: 100,000,000 the effects begin to ap-

pear in ten days, but with a solution 1: 1,000,000 the horny jaws are completely lost at the end of four days. We have here a very ready response to thyroxin.

Measurements of the length of the tongue were undertaken, but the small size, irregular form, and rotation in position make an accurate evaluation very difficult so they were discontinued. The following statements may be made. There was little evidence of the tongue during the ten days of the experiment in the controls and in the 1: 1,000,000,000 solution although the anlage was visible. In the solutions of



Graph. 8 Experiment 10. Bufo halophilus. Growth of tongue. See legend of graph 3.

1: 100,000,000; 1: 10,000,000, and 1: 1,000,000 it appeared on the fourth day (graph 8) and grew quite rapidly, roughly in proportion to the concentration of the thyroxin solution.

The color of the gall bladder gives interesting data, especially because it indicates a stage in physiological activity of the liver. In the controls and 1: 1,000,000,000 thyroxin solution it remained white through the duration of the experiment (graph 9), while in the 1: 100,000,000 solution, one of the ten specimens showed a green gall bladder on the eighth day. In the 1: 10,000,000 solution one out of ten showed the green

color on the fourth day, while in the 1: 1,000,000 solution nine out of ten showed the green color on the fourth day. Unfortunately, observations were not made upon this point in all of the lots, but the general facts are obvious enough and perhaps all that we can expect from criteria based upon a tint of color.

GRAPH 9

Experiment 10. Change in color of gall bladder

Control	W 10	W 10	W 9	W 8	W 10
1: 1,000,000,000	W 10	W 10	W 10	W 10	W 10
1: 100,000,000	W 10	W 10	W 10	W 9 G 1	
1: 10,000,000	W 10	W 9 G 1	W 4 G 6	W 2 G 7	W 4 G 6
1: 1,000,000		W 1 G 9	W 6 G 4		
	2 D.	4 D.	6 D.	8 D.	10 D.

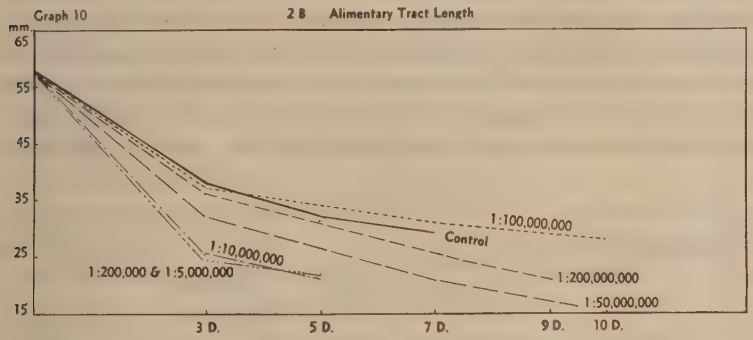
Graph 9 Experiment 10. Bufo halophilus. Change in color of gall bladder. W indicates white color, G indicates green color. See graph 3.

Experiment 2B

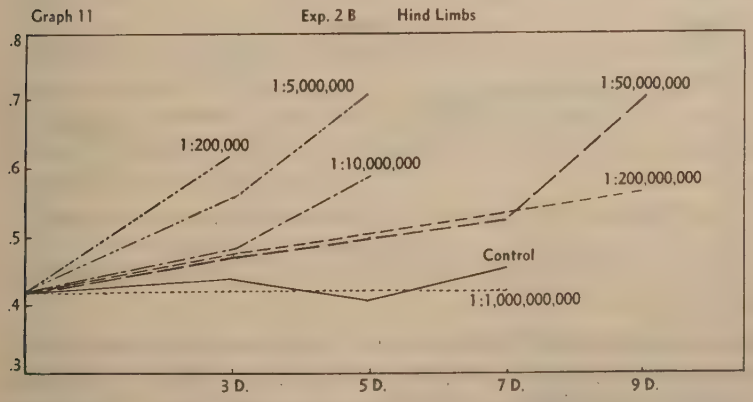
In this experiment we used a very well matched lot of tadpoles with hind limbs 0.35 to 0.50 mm. in length, and with a total length of 19.0 to 22.0 mm. The concentrations employed were 1: 1,000,000,000, 1: 200,000,000, 1: 50,000,000, 1: 10,000,000, 1: 5,000,000, and 1: 200,000, representing a wide range in which the strongest solution was 5000 times as concentrated as the weakest. The time intervals chosen were three, five, seven, and in some cases ten days after immersion in the solution. Graph 4 gives the results of this experiment.

The alimentary tract undergoes shortening in response to the action of thyroxin in a concentration of 1: 200,000,000 (graph 10). This is well marked in the course of seven days. The more concentrated solutions show a graded influence corresponding to the concentration, culminating in the 1: 5,000,000 solution that reduced the length of the alimentary tract

to two-thirds that of the control at the end of three days. This appears to be very near the maximum effect because it was not exceeded by the 1: 200,000 solution.



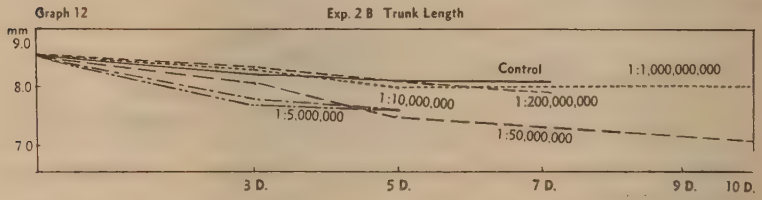
Graph 10 Experiment 2B. Bufo halophilus. Alimentary tract length. See legend of graph 3.



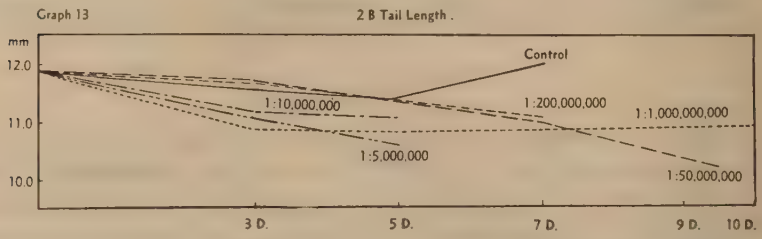
Graph 11 Experiment 2B. Bufo halophilus. Hind limbs. See legend of graph 3.

The hind leg is clearly increased in length 20 per cent beyond normal in the 1: 200,000,000 solution in the course of seven days. This growth is exceeded strikingly in the stronger concentrations as shown in graph 11.

At the end of five days our curves show little difference between the growth of the tail in the controls and in the thyroxin solutions, except in the case of the 1:5,000,000 solution where a shortening is evident. At the end of nine to ten days the tadpoles in the solution of 1:50,000,000 show a decided diminution in the length of the tail. In general it may be said that the tail length is the least reliable of all the criteria for development. The dip in the curve for the control and its subsequent rise illustrate this point. We may



Graph 12 Experiment 2B. Bufo halophilus. Trunk length. See legend of graph 3.



Graph 13. Experiment 2B. Bufo halophilus. Tail length. See legend of graph 3.

similarly interpret the low level of the curve representing the tail length in the 1:1,000,000,000 tadpoles. These specimens chanced to have shorter tails than the other lots. It is, however, significant that there is no decrease in the length at the end of ten days. In this series the tadpoles had not reached a stage at which the tail had shown much capacity to respond to the influence of thyroxin.

There is a fairly definite shortening of the trunk in concentrations of 1:50,000,000, 1:10,000,000, and 1:5,000,000 as can be seen by reference to graph 12.

DISCUSSION

It is seen that the alimentary tract gives a quick and certain response to the action of thyroxin. Its rather excessive length at the start of 2B is due to the fact that the tadpoles in the initial stage of this set were killed soon after being brought into the laboratory instead of being held for some time as in the case of experiment 10. The differences in the effect of the different solutions are quite clear on the second and third day. While the influence of a concentration of 1:200,000,000 becomes evident on the fifth day, the stronger solutions give very early changes.

This principle of the difference in the time of first appearance of a response proportional to the concentration of the solution is evident in all of the structures studied. The threshold of response differs in different structures when measured at the end of a stated period, as six days in the case of experiment 2A, graph 2. At the end of this period the alimentary tract and hind limbs are found to be affected by solutions of 1:100,000,000 and even by solutions of 1,200,000,000 in the case of the hind limbs. Within this time the tail responds only to solutions as strong as 1:5,000,000—a relatively high concentration. A solution of 1:1,000,000 produces a sharp shortening of the tail before the fourth day, as shown by experiment 10, graph 4.

Data on the shortening of the trunk are not so satisfactory as the other features under consideration, because the difficulty of making accurate measures involving the cloacal opening, which is membranous and situated out at a slight distance from the base of the tail. In experiment 2B, a solution of 1:50,000,000 produced a shortening of this measurement on the fifth day and in the two other experiments effects at this time were produced only by greater concentrations, 1:5,000,000. In general, we may say that the trunk length is less easily affected than most of the other features.

Changes in the color of the gall bladder were studied only in experiment 10. It was there shown that this change is induced by a concentration of 1:100,000,000 at the end of

eight days and in a concentration of 1:1,000,000 was very marked on the fourth day.

Tongue growth was studied in experiments 2A and 10. In the former, a distinct effect was recorded in response to the solution of 1:1,000,000 at the six-day termination of the experiment, while results were seen in experiment 10 at the end of four days in a concentration of 1:100,000,000. In this experiment at least, the tongue was seen to be fairly responsive to the thyroid hormone.

The loss of the horny jaws observed in the same two experiments, seemed to follow much the same course as above, although in experiment 10 this process was first evident on the tenth day in the 1:100,000,000 solution.

Other observations not included in the scope of this paper showed that almost complete metamorphosis can be attained in a solution of 1:200,000,000 of thyroxin in a sufficiently extended period of time. The time element is sufficiently in evidence in the data here given to establish the fact that certain structures respond quickly in high concentrations and more tardily in weak concentrations. The threshold of stimulation discussed by Blacher must be considered in this light. By the conditions of his experiments, it is a response within a given period of time, but we have here shown that weak solutions probably far below this threshold limits produce decided effects if given a sufficient period of time in which to operate. Keeping this point in mind, if we choose five or six days as a period of duration of the experiment and arrange the different structures in the order of their sensitiveness to thyroxin as applied in different concentrations, they could show the following 'thresholds of stimulation' if rated in the order of their ability to respond at this time: 1) alimentary tract, 2) hind limbs, 3) change in color of gall bladder, tongue growth, loss of horny jaws, 4) reduction in length of tail, reduction in length of body. Because of the difficulty of making finer distinctions from the data at hand, this union of several structures in groups three and four is made. The principle of different degrees of sensitiveness is clear and in

this we concur with Blacher ('28). He used older stages in his experiments. His order of threshold of response as determined at the end of three days immersion was: 1) intestine, 2) tail, 3) trunk, 4) loss of horny jaws, 5) fore limbs.

We do not wish to stress the differences in our work with that of Blacher. His experiments were made at a stage much nearer that for the shortening of the tail and trunk and development of fore limbs than were ours. We may also point out the fact that he killed his specimens at the end of three days, whereas our own continued for six to even ten days. It is furthermore difficult to compare the strength of the thyroid hormone stimulus in his work and in ours, because Blacher used thyroidin while we used thyroxin.

A study of the results of experiment 2C made upon very young tadpoles beginning in the tail bud stage and continued for eighteen days, shows that certain changes may be induced at these early stages, but they are slight in amount and slow in development. This is very marked in comparison with the changes induced in the other experiments begun in tadpoles with an average total length of 20 mm.

We are in agreement with Blacher upon the point that there is a difference in the response of different structures at the end of a given relatively short period of time. During this period, certain structures respond to weak solutions of the thyroid hormone, whereas others require a much higher concentration in order to respond during the specified time and the responsiveness of the different parts in our work corresponds in many regards with the findings of Blacher.

Our experiments show that this 'threshold of response' is really a question of 'time of response.' Structures that do not develop as quickly as others in weak solutions will respond in these same solutions after longer exposure to them. It seems that our data leave little doubt upon this point.

SUMMARY

1. Tadpoles of Bufo halophilus at a stage well prior to the rapid changes of metamorphosis were exposed to thyroxin solutions ranging in concentration from 1: 200,000 to 1: 1,000,000,000 (5000 times more dilute).

2. No response of any portions is observed at solutions of thyroxin of concentrations less than 1: 200,000,000 and in this solution the response during the time limits of these experiments is very slight.

3. The different parts studied may be arranged in order of responsiveness to lower concentrations of thyroxin as follows: 1) alimentary tract, 2) hind limb, 3) change in color of gall bladder, tongue growth, loss of horny jaws, 4) reduction in length of tail, reduction in length of body.

4. The speed of response is in proportion to the concentration of the thyroxin solution used up to certain maximum limits, beyond which greater concentrations cannot give greater rate of development.

5. The influence of thyroxin is proportional to the age of the tadpole, being slight in the young periods beginning with tail bud stage and becoming far more pronounced in the period of 20 mm. total length.

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THE DOMINANT RÔLE OF THE PARS ANTERIOR OF THE HYPOPHYSIS IN INITIATING AMPHIBIAN METAMORPHOSIS[1]

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SIX FIGURES

Twelve years ago Allen ('20) showed that addition of the pars anterior of the adult hypophysis to hypophysectomized tadpoles enables the thyroid gland to function. It had been previously shown that the thyroid gland would not function in the absence of the hypophysis and this experiment demonstrated first that the pars anterior is the portion involved, and secondly that the activity of the hypophysis can begin after a long initial period of arrest. These experiments also demonstrated (Allen, '21) that persistent transplants of large pieces of healthy adult hypophysis substance fail to induce the slightest tendency to metamorphosis in thyroidectomized tadpoles even when the transplants remain in healthy and presumably active condition for periods of four or five months.

Allen ('21 and '27) made a more detailed analysis of the function of pars anterior, pars intermedia, and pars nervosa by the same method of transplantation, showing that the addition of the pars anterior causes decided growth of the follicles of thyroidectomized tadpoles.

Ingram ('28) induced metamorphosis of the slowly metamorphosing tadpoles of Rana catesbiana by transplanting

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It is a pleasure to express my deep appreciation for the pleasant laboratory quarters placed at my disposal through the kindness of the Del Amo Foundation.

into them the pars anterior of the hypophysis from the rapidly developing species, Rana pipiens. In a more extensive investigation (Ingram, '29) upon the influence of pars anterior material of Rana pipiens transplanted into the slowly developing Rana clamitans, employed the same type of experiment. He employed the technique of placing the transplant in the body cavity. From the standpoint of our present paper, the most interesting part of his work is that in which he transplants the hypophysis of larvae. But he has not attacked the problem that we are trying to solve, because he does not take into account the degree of development of the donors, and his method is to transplant as many as twenty glands into the body cavity of each tadpole at different times during the course of the experiment. Many of these transplants are lost through the opening of the body wall into which they have been thrust. He has not attacked the problem of the stage of development at which the pars anterior becomes effective, nor has he aimed at a quantitative study.

He finds that the thyroid glands of the experimental animals undergo a decided increase in size as compared with those of the controls, and that the follicle cells show a tendency to lengthen, taking on the form that characterizes them in the active gland. There also appears to be considerable discharge of colloid substance from the follicles of the experimental animals.

In a later paper ('30) Ingram dealt with the Golgi apparatus of the follicle cells of the thyroid gland in tadpoles into which pars anterior transplants had been placed as above. He found this apparatus to be well developed, an evidence of greater activity of the cells, but he does not find evidence of a correspondence between polarity of the Golgi apparatus and the direction of secretion.

Grant ('30) has found this same release of colloid from the follicles of the thyroid gland of Necturus maculosus larvae of 45-mm. length under the influence of intraperitoneal transplants of the pars anterior of the hypophysis of adult Rana pipiens. In a later paper ('31) Grant gave the results of

similar work in which the discharge of the colloid from thyroid follicles of Amblystoma jeffersonianum was described in detail. In these experiments, portions of the pars anterior of the hypophysis of different species of frogs were daily transplanted into the peritoneal cavity. Studies were made at intervals of one to seven days and decided effects recorded.

Our problem in the present paper is to test the influence exerted by the transplants of the pars anterior of the pars anterior of the hypophysis taken from tadpoles of different stages of development. In such work it is essential to choose for host animals to receive the transplants a stage prior to the appearance of thyroid activity and yet close enough to that period so that there will be ready response to the influence of the transplant. In order that this work may have quantitative value, the transplants must be accurately accounted for and checked up by dissection and microscopic study at the end of the experiment.

MATERIAL AND METHODS

Our method of attack was to transplant the pars anterior of the hypophysis of Bufo halophilus from tadpoles in various stages of development, recently metamorphosed toads, and adults into young tadpole hosts of the same species, in which the hind limbs had attained a length of 1 mm. In some cases, designated as '31—2½ C, M, O, P, and E, the recipients or hosts were slightly more advanced at the time of operation, having hind legs 2 to 3 mm. long. These latter usually responded more quickly to the influence of the transplant than did the younger hosts, and yet the development of the controls was likewise rapid—so they did not serve so well as a test of our problem as did the younger hosts.

Our method was to make a pocket under the skin of the top of the head immediately median and posterior to the right eye and into this insert the pars anterior of the hypophysis of the donor stage to be tested. In some instances the pars intermedia and pars nervosa were also included, but since these have been shown by our previous experiments to have

no influence upon metamorphosis either by stimulation or retardation, they may be ignored. Healing takes place very quickly, and the course of the transplant can be fairly well detected through the skin. In all cases it was removed for study after the termination of the experiment. Vascularization could be readily detected by gross observation with a dissecting microscope. In each instance a camera-lucida drawing of the transplant was made for purposes of comparison. Typical specimens were sectioned in order to determine the histological character of the transplant. With the exception of a few cases, the transplants were found in good condition, and these formed the basis of our studies.

The sets of tadpoles were killed at the end of ten, fifteen, and eighteen days, and preserved in Bouin's fluid for study. Determinations were made of: 1) total length, 2) tail length, 3) hind-leg length. The number of tadpoles of each lot varied from five to fifteen, from which a reasonably accurate average could be computed—quite reliable in numbers above ten.

These experiments were performed in the summers of 1930 and 1931, and the specimens used amounted in all to 219 experimentals and 111 controls. In a number of cases one set of controls could be used for several types of transplant made on the same day or within a day of the operations.

In order to meet the objection that the difference of influence of the pars anterior of the hypophysis of different-aged donors could be due to differences in size alone, we made double transplants of the hypophysis in the case of donors with hind limbs of less than 3-mm. length. In all other cases single transplants were employed.

RESULTS

A study of the tables of measurements clearly shows that growth in length of the hind limbs and shrinkage in the tail do not occur in any consistent degree where the hypophysis, even in double dose, is taken from donors with hind legs 3 mm. or less in length. No significant effect was produced by transplants from donors with hind limbs of 5 to 8 mm. total length,

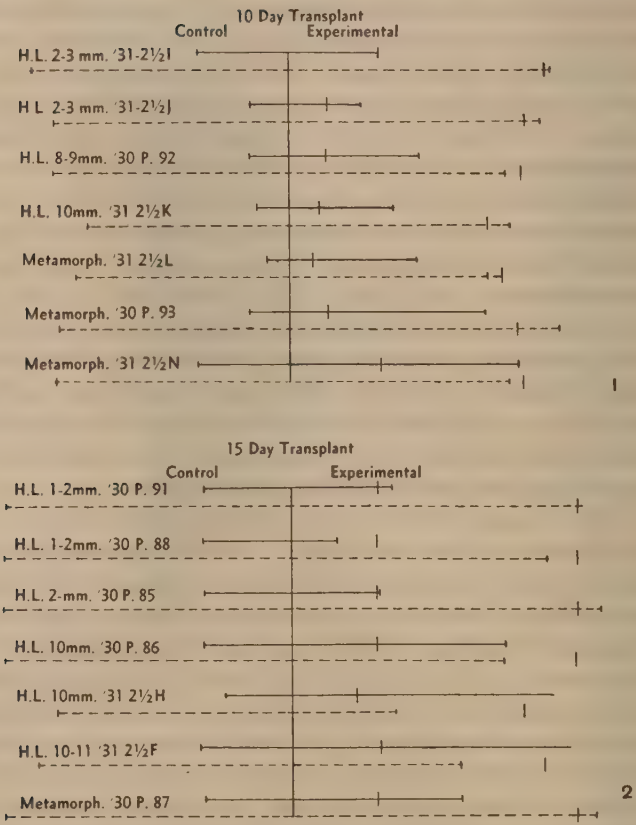
ranging nearer 5 than 8. This was found to be true as determined by fore-limb and tail length of the recipients in experiment no. '30 P 81, which ran for a period of eighteen days.

Transplants from donors with hind limbs 6 to 11 mm. length ('31-2½ O) and from those of 8 to 9 mm. hind-limb length ('30 P 92) produced a pronounced acceleration of metamorphosis as indicated by the above-mentioned criteria.

Efforts were made to determine whether there were differences in the effects of transplants from donors with hind limbs of greater length than these, namely, 10 mm., 12 mm., metamorphosed, and adult. The differences in the effects produced by these different later stages in the development of the anterior hypophysis were not accurate enough to give a clear distinction between them, nor could this be attained save by the use of very large numbers, and the careful elimination of all in which the transplants do not measure up to quantitative standards of good preservation.

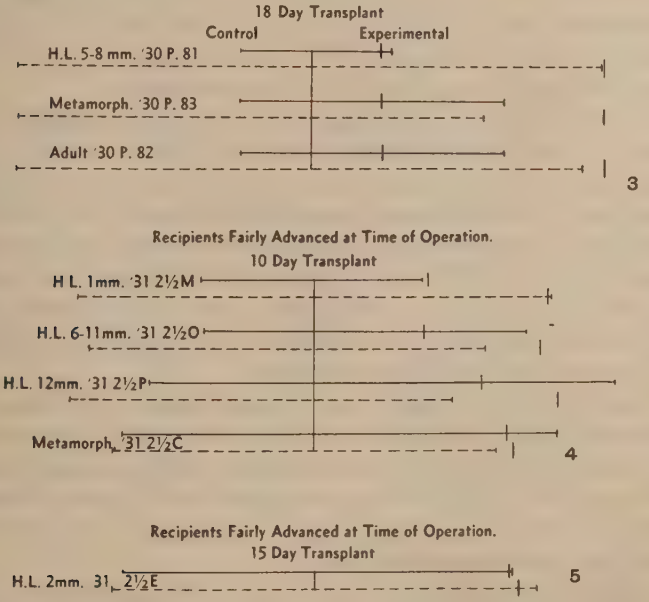
It will be seen that the fore limbs likewise develop under the influence of the transplant. Their response is next in degree to that of the hind limbs, so that we may arrange these different criteria in the order of their responsiveness to the pars anterior of the hypophysis as follows: 1) hind limbs—growth, 2) fore limbs—growth, 3) tail—reduction.

These points are graphically shown in the diagrammatic illustrations (figs. 1 to 5), the solid lines representing the average lengths of the hind limbs of the tadpoles at the termination of the experiment, and the broken lines similarly representing the average lengths of the tails in the given experiments. Each of these lines is cut by the vertical base line; the portion to the left indicates these dimensions in the controls. On the portion of the line to the right, representing the experimental groups, we have marked off these dimensions of the corresponding controls—the difference being thus brought out in obvious fashion. Lines indicating hind-leg length are drawn to a scale of 1 to 8, while those indicating tail length are made upon a scale of 1 to 4. This was done in order to bring the two elements within the same space limits of the diagram.



Figs. 1 to 5 Solid lines represent the hind limb length, × 6. Broken lines represent the tail length, × 3. The portions of the lines to the right of the vertical base line indicate the averages of experimental groups, while the portions to the left indicate the averages of their corresponding controls. Each 'control' segment is projected on its 'experimental' segment as indicated by the cross line which may either intersect the 'experimental' segment or may lie beyond it. These values represent averages of lots of ten tadpoles, sometimes slightly less. The tadpole 'experimental' groups are labeled at the left and are classified according to the character of the donors from which the transplants are taken. *H.L.* indicates hind-limb length as noted in each case. '*Metamorph*' signifies that the transplants were secured from recently metamorphosed animals.

Comparison of the diagrams bears out the general statements made above. There are certain irregularities in the results that neutralize one another, as in the case of '31-2½ J compared with '30 P. 88. Slight differences in the specimens at the beginning of the experiment account for these cases. In other cases of the young donors, as '31-2½ I, '30 P. 91, and '30 P. 85, the correspondence in experimental and control lots is strikingly close.



In the case of the line indicating hind-leg length the experimental lots show a greater length than the corresponding control lots if the transplants are effective in inducing a tendency to metamorphosis. On the other hand, the reverse is true of the lines indicating tail length because metamorphosis involves a decrease in its length. The experiments in which hosts of a fairly young stage of development, 2 to 3 mm. hind-limb length, were used, the hind limbs responded to the

influence of the transplant far more readily than the tail, as will be seen at a glance in the diagrams. On the other hand, those experiments in which the hosts were fairly advanced at the beginning of the experiment—hind limbs 6 to 8 mm. as in '31-2½ O, '31-2½ P, and, to a less degree, '31-2½C, there was a distinct effect upon the tail even during the ten days of these experiments. Such an effect upon the tail is occasionally seen in the fifteen- and eighteen-day experiments, even in cases where the development at the beginning of the experiment was not so great, as in '30 P. 86, '31-2½ H, and '31-2½ F, also in '30 P. 83. We can account for this on the basis of longer time available for development.

A general survey of these graphs in which so many, especially in the ten-day series, show a decided growth of the hind limbs with no reduction in the tail, leads to the conclusion that the hind limbs respond more readily to the transplant than does the tail, especially so in the earlier stages. This is in line with our experiments upon the influence of thyroxin and it is to be expected, since the hind limb begin to show the growth characteristic of metamorphosis before the resorption of the tail commences.

Figure 6 gives a clear idea of the differences resulting from these different types of transplants. While such observations of 'general appearance' may not give such accurate conclusions as the study of measurements that we have discussed above, yet they are rather more convincing in that the differences are apparent at a glance.

We have given some attention to a histological explanation of this question. Studies have been made upon the relative number and degree of development of the eosinophile and basophile cells of the pars anterior of the hypophysis of the donor types. It is quite certain that the eosinophile cells develop before the basophile cells. In studies of stages with a hind-limb length of 1.4 mm. both types are present, but the eosinophile cells are relatively much more numerous at this stage than at the period when the hind limbs have reached a length of 9 mm. More significant still is the fact that the

eosinophile cells may be deeply stained, even in these early stages, while the basophile cells stain very faintly. Observa-

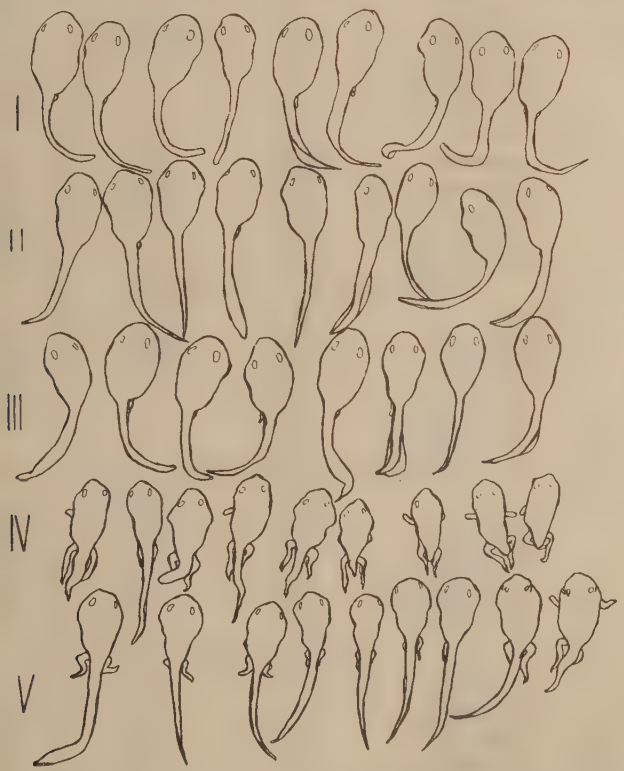


Fig. 6 Outline drawings of tadpoles of Bufo halophilus at end of stated periods after receiving transplants of the pars anterior of the hypophysis of different donor stages. I. Controls at end of eighteen days. II. Recipients of transplants from donors with hind limbs 2 mm. long. At end of fifteen days. III. Recipients of transplants from donors with hind limbs 5 to 8 mm. long. At end of eighteen days. IV. Recipients of transplants from donors with hind limbs 10 mm. long. At end of fifteen days. V. Recipients of transplants from donors that had recently metamorphosed. At end of ten days.

tions of this kind would be very inconclusive were it not for the fact that we can compare the basophile cells of the pars anterior with the cells of the pars intermedia in the same

TABLE 1

Ten-day transplant

		EXPERIMENTAL, IN MM.	CONTROL, IN MM.	DIFFERENCE, IN MM.
Donor hind leg 2 to 3 mm. (double transplant)				
'31-2½ I	Number	9	5	
	Total length	26.32	25.96	0.36
	Trunk length	11.15	11.10	0.05
	Tail length	15.17	14.86	0.31
	Hind-leg length	2.60	2.68	0.08
	Fore leg length	0	0	
'31-2½ J	Number	15	10	
	Total length	25.74	23.95	1.79
	Trunk length	11.18	10.29	0.89
	Tail length	14.56	13.66	0.90
	Hind-leg length	2.07	1.15	0.82
	Fore leg length	0	0	0
Donor hind leg 8 to 9 mm. length				
'30 p 92	Number	9	10	
	Total length	22.44	23.95	1.51
	Trunk length	9.94	10.29	0.35
	Tail length	12.50	13.66	1.16
	Hind-leg length	4.92	1.15	3.77
	Fore leg length	(1)6 (2)2		
Donor hind leg 10 mm., 4 limbs, tail 4 to 6 mm.				
'31-2½ K.	Number	15	14	
	Total length	21.44	20.76	0.68
	Trunk length	8.64	9.12	1.48
	Tail length	12.80	11.64	1.16
	Hind-leg length	3.02	0.95	2.07
	Fore leg length	(1)1	0	
Donor metamorphosed				
'31-2½ L.	Number	12	17	
	Total length	21.53	20.65	0.88
	Trunk length	9.15	8.26	0.89
	Tail length	12.38	12.39	0.01
	Hind-leg length	3.07	0.67	2.40
	Fore leg length	(1)8 (2)1	0	
'30 p 93	Number	8	10	
	Total length	26.24	23.95	2.29
	Trunk length	10.80	10.29	0.51
	Tail length	15.44	13.36	2.08
	Hind-leg length	5.64	1.15	4.49
	Fore leg length	(1)4	0	
'31--2½N.	Number	13	6	
	Total length	22.6	24.5	1.9
	Trunk length	9.9	10.9	1.0
	Tail length	12.7	13.6	0.9
	Hind-leg length	6.65	2.7	3.97
	Fore leg length	(2)4 (1)4 (0)5	0	

TABLE 2

Fifteen-day transplant

		EXPERIMENTAL, IN MM.	CONTROL, IN MM.	DIFFERENCE, IN MM.
	Donor hind leg 1 to 2 mm. length at operation			
'30 p. 91	Number	10	5	
	Total length	29.50	28.52	0.98
	Trunk length	12.62	11.78	0.84
	Tail length	16.88	16.74	0.12
	Hind-leg length	2.95	2.54	0.41
	Fore-leg length	0	(2)2	
	Donor hind leg 1 to 2 mm. length at operation			
'30 p. 88	Number	10	5	
	Total length	26.45	28.52	2.07
	Trunk length	11.54	11.78	0.24
	Tail length	14.91	16.74	1.83
	Hind-leg length	1.30	2.54	1.24
	Fore-leg length	0	0	
	Donor hind leg less than 2 mm. length at operation			
'30 p. 85	Number	10	5	
	Total length	31.20	11.78	2.68
	Trunk length	12.42	28.52	0.64
	Tail length	18.78	16.74	2.04
	Hind-leg length	2.57	2.54	0.03
	Fore-leg length	0	0	
	Donor hind leg 10 mm. length at operation			
'30 p. 86	Number	9	5	
	Total length	23.80	28.52	4.72
	Trunk length	11.50	11.78	0.28
	Tail length	12.30	16.74	4.44
	Hind-leg length	6.23	2.54	3.69
	Fore-leg length	(2)5 (1)1	0	
	Donor hind leg 10 mm. length at operation			
'31-2½ H	Number	14	21	
	Total length	15.64	24.70	9.06
	Trunk length	9.59	11.04	1.45
	Tail length	6.05	13.66	7.61
	Hind-leg length	7.56	1.93	5.63
	Fore-leg length	(2)11 (1)1		
	Donor hind leg 10 to 11 mm. length at operation			
'31-2½ F	Number	9	3	
	Total length	19.33	24.80	5.47
	Trunk length	9.52	11.00	0.48
	Tail length	9.81	13.80	3.99
	Hind-leg length	8.17	2.67	5.50
	Fore-leg length	(2)7 (1)2	0	
	Donor metamorphosed			
'30 p. 87	Number	10	5	
	Total length	28.33	28.52	0.19
	Trunk length	11.73	11.78	0.05
	Tail length	17.60	16.74	0.86
	Hind-leg length	4.91	2.54	2.37
	Fore-leg length	(2)1 (1)2	0	
	Host fairly advanced at time of operation. Donor hind leg 2 to 2.5 mm.			
'31-2½ E	Number	12	7	
	Total length	24.40	22.91	1.49
	Trunk length	11.46	11.04	.42
	Tail length	12.94	11.87	1.07
	Hind-leg length	5.77	5.67	0.10
	Fore-leg length	(2)4	(2)2	

sections, and it is quite evident that they are relatively faint in the early stages and very much more deeply stained at a later period.

We are proceeding with studies along these lines, and the above remarks should be considered tentative until more

TABLE 3

Eighteen-day transplant

		EXPERIMENTAL, IN MM.	CONTROL, IN MM.	DIFFERENCE, IN MM.
	Donor hind leg 5 to 8 mm.			
'30 p. 81	Number	8	9	
	Total length	30.05	30.47	0.42
	Trunk length	13.09	13.21	0.12
	Tail length	16.96	17.26	0.30
	Hind-leg length	2.34	2.09	0.25
	Fore-leg length	0	0	
	Donor at metamorphosis			
'30 p. 83	Number	9	9	
	Total length	20.92	30.47	9.55
	Trunk length	10.88	13.21	2.33
	Tail length	10.04	17.26	7.22
	Hind-leg length	5.61	2.09	3.52
	Fore-leg length	0	0	
	Donor adult one-eighth part proximo			
'30 p. 82	Number	10	9	
	Total length	25.47	30.47	5.0
	Trunk length	10.65	13.21	2.56
	Tail length	15.82	17.26	1.40
	Hind-leg length	5.63	2.09	3.54
	Fore-leg length	0	0	

In entire investigation:

Experimentals 219
Controls 111

thorough studies shall have been made. Work of this type is difficult and entails a vast amount of careful study. These facts are at least suggestive.

Table 5 shows the measurements of the dissected thyroid glands as determined by use of a planimeter applied to camera-lucida drawings. These two dimensional measurements do not tell the whole story because there are large and

significant differences of thickness. The measurement of this dimension would be extremely laborious and hardly worth the labor. One can see at a glance that transplantation of the pars anterior of the hypophysis from donors in process

TABLE 4

Ten-day transplant. Recipients fairly advanced at time of operation

		EXPERIMENTAL, IN MM.	CONTROL, IN MM.	DIFFERENCE, IN MM.
Donor hind leg 1 mm. (double implant)				
'31-2½ M.	Number	11	8	
	Total length	24.9	25.2	—00.3
	Trunk length	11.0	11.4	—00.4
	Tail length	13.9	13.8	
	Hind-leg length	3.15	3.32	— 0.17
	Fore-leg length	0	0	
Donor hind leg 2 to 2.5 mm. (double implant)				
'31-2½ E	Number	12	7	
	Total length	24.40	22.91	+01.49
	Trunk length	11.46	11.04	+00.42
	Tail length	12.94	11.87	
	Hind-leg length	5.77	5.67	+ 0.10
	Fore-leg length	(2)4	(2)2	
Donor hind leg 6 to 11 mm.				
'31-2½ O	Number	13	12	
	Total length	20.02	23.94	—03.92
	Trunk length	11.04	10.61	+00.43
	Tail length	9.98	13.33	
	Hind-leg length	6.16	3.24	+ 2.92
	Fore-leg length	(2)5 (1)1	0	
Donor hind leg 12 mm.				
'31-2½ P	Number	8	7	
	Total length	18.66	25.72	—07.06
	Trunk length	10.72	11.39	—00.67
	Tail length	7.94	14.33	
	Hind-leg length	8.76	4.82	+ 3.94
	Fore-leg length	(2)8	0	
Donor metamorphosed				
'31-2½ C	Number	8	7	
	Total length	20.92	22.91	—01.99
	Trunk length	10.30	11.04	—00.74
	Tail length	10.62	11.87	
	Hind-leg length	7.10	5.67	+ 1.43
	Fore-leg length	(2)6	(2)2	

of active development (hind-leg length 8 to 10 mm.) and the stage of completed metamorphosis, has caused the thyroid glands of the recipient to grow in size. Whatever may be the function of the pars anterior as a releasing mechanism, it causes the gland to enlarge. With small numbers of speci-

TABLE 5

Showing the influence of transplants of the pars anterior of the hypophysis upon the thyroid glands of the recipients

	THYROID AREA, BOTH GLANDS	HIND LEG, MM.	NUMBER OF SPECIMENS	DAYS OF EXPERIMENT
Controls				
'30 P. 80	211	2.09	9	15
'30 P. 84	179	2.54	5	15
Donors				
Hind leg 1 to 2 mm.				
'30 P. 91	194	2.43	10	10
'30 P. 88	152	1.30	10	15
Hind leg 5 to 8 mm.				
'30 P. 81	202	2.34	8	18
Hind leg 8 to 9 mm.				
'30 P. 92	262	4.92	8	10
Hind leg 10 mm.				
'30 P. 86	279	6.23	9	15
'30 P. 83	227	5.61	9	18
Metamorphosed				
'30 P. 93	253	5.64	8	10
'30 P. 87	285	4.91	9	15
Adult				
'30 P. 82	204	5.63	9	18

mens, a few advanced specimens chosen for the experiments make irregularities in these data, but the general trend of the figures is clear enough.

The follicles of the thyroid glands of the experimental specimens whose development has been stimulated by anterior hypophysis contained solid masses of colloid in the distended follicles, so we are forced to the conclusion that the transplants induce the deposition of colloid in the thyroid

follicles. We have abundant material for the study of the function of the pars anterior of the hypophysis, and it is our intention to test the view that it acts as a 'releasing mechanism' for the thyroid gland. Much of our material is sectioned and ready for study, but we prefer to reserve judgment upon this phase of the problem pending more crucial experiments.

CONCLUSIONS

In the great majority of these experiments we are dealing with tadpoles in which the process of metamorphosis has not yet commenced. It is true the hind limbs have begun to develop, having attained a length of 2 to 3 mm., but they have not yet reached the degree of differentiation and length in proportion to body length shown by the hind limbs of thyroidectomized or hypophysectomized tadpoles at maximum development. Both thyroid gland and hypophysis are present, but are not producing secretion of sufficient quality or quantity to bring about metamorphosis. Previous work has well shown that the thyroid gland does not produce effective secretion unless the pars anterior of the hypophysis is present. In 1920 we showed that transplantation of the pars anterior of the adult in very large quantities is not capable of inducing metamorphosis in thyroidectomized tadpoles—that no matter how great the secretion of the pars anterior of the hypophysis, it can only operate through the thyroid gland.

The recipients of our transplants have small, poorly developed thyroid glands with meager accumulations of colloid in the follicles, but it does not necessarily follow that they contain thyroxin. In any case, development has not proceeded to a point where the thyroid function comes into play. We make a transplant of the pars anterior of the hypophysis taken from a tadpole in process of metamorphosis, and as a result, metamorphosis begins in the tadpole that receives it, while there is no sign of such process in the controls.

We conclude that the pars anterior of the hypophysis plays the leading rôle in initiating metamorphosis. Although the thyroid secretion is stored up in the thyroid gland, it is quite possible that the pars anterior of the hypophysis may play an important, perhaps a dominant, rôle in the manufacture of the thyroid hormone.

It might be interesting to speculate as to whether we could transplant an advanced thyroid gland into a young tadpole of this recipient stage and thus induce the functioning of the pars anterior of the hypophysis. Perhaps means may be found to test this question, but our procedure could not follow the lines of this work, because of the fact that the stored secretion might be the effective factor.

We are not dealing here with a 'releasing mechanism,' or, at least not to any considerable degree; but find, on the other hand, that the pars anterior of the hypophysis initiates the production of the secretion, although it is quite possible to conceive that it could serve the function of a releasing mechanism if any considerable quantity of thyroid colloid were present.

SUMMARY

1. The pars anterior of the hypophysis was removed from tadpoles of Bufo halophilus of various stages and transplanted under the skin of the top of the head of tadpoles of a stage of 1 mm. hind-leg length (in some cases 2 and 3 mm. length). When taken from donors in process of metamorphosis or in which that process had been completed, the transplant induced development toward metamorphosis in the recipient.

2. The effect upon leg development appeared before the other effects, to be followed later by development of the fore limbs and by shortening of the tail, in the order named.

3. These differences in action of the transplants were not due to differences in bulk of transplanted gland substance as demonstrated by use of double amounts from younger tadpole donors. Preliminary studies showed that the basophile cells, although present in these early stages, are few in number

and stain faintly. This is in contrast to the much greater development of the eosinophile cells. These observations appear to support the view that the basophile cells are the ones responsible for inducing metamorphosis by cooperation with the thyroid gland.

4. Transplants of the pars anterior of the hypophysis taken from tadpoles during the process of active metamorphosis or at completion of that process induce increase in size of the thyroid glands of the experimental tadpoles into which they are transplanted and consequently hasten their metamorphosis. We can conclude that the pars anterior of the hypophysis takes a leading part in initiating metamorphosis.

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A CASE OF A CLOSED CORONARY SINUS AND LEFT SUPERIOR VENA CAVA

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ONE FIGURE

Bilateral superior venae cavae in man are not such infrequent anomalies as we might expect. In fact, K. Kodama ('31), in a recent report of a case, has pointed out that 138 cases of bilateral superior venae cavae have been listed by R. Locchi ('27) from European literature. Where two superior venae cavae are present, they may be equal in size and in function, but frequently the unusual left replaces a defective right. However, the occurrence of a closed coronary sinus in such cases is a rather rare anomaly, as a review of the literature reveals. Beyerlein ('14) described a case of this type in which the left superior vena cava drained the blood from the cardiac veins and Hutton ('15) reported a similar case. Both of these workers have discussed the condition from the viewpoint of its origin during development. They also referred to descriptions of two other cases in earlier literature, one by Gruber ('85) and one by Le Cat in 1738. Because of the relative rarity of this anomaly, a fifth case recently observed in our laboratory is described below.

The heart (fig. 1) was found by students dissecting the body of an adult negro male, the cause of whose death was not ascertained. The heart was not unusual as to size, shape, or position, nor were there any irregularities in the arrangement of the great vessels other than the presence of the left superior vena cava. The arteries of the heart were not unusual, and while the cardiac veins were prominent, they

drained into the coronary sinus without much variation from the normal arrangement.

The coronary sinus was about 3 cm. in length, was considerably dilated, and showed only a very small connection (X in the figure) with the right auricle. The middle and

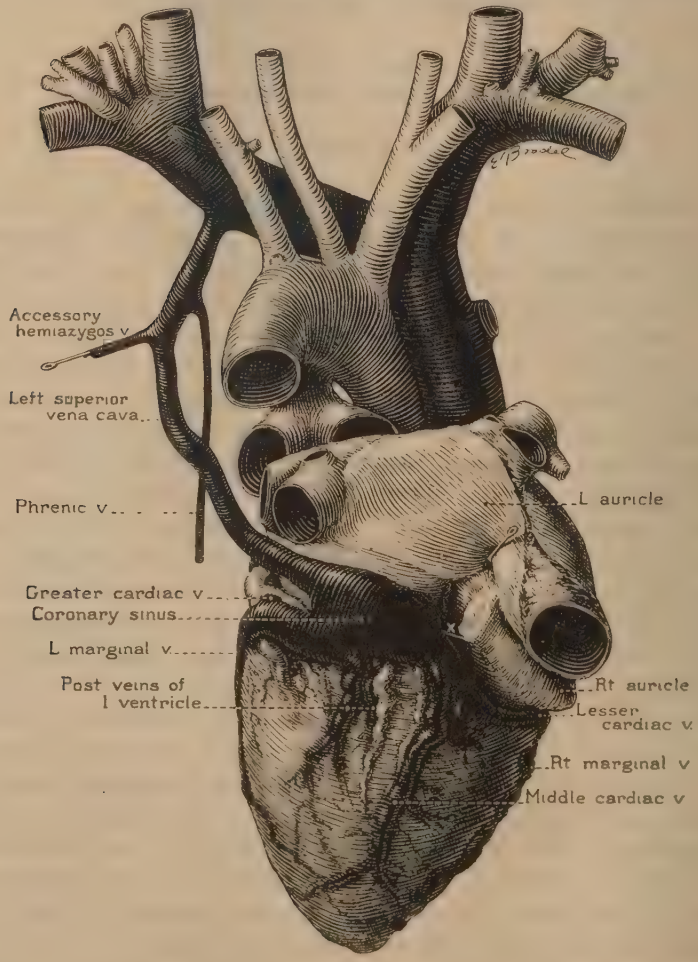

Figure 1

lesser cardiac veins emptied into the sinus together, while opposite their orifice the greater cardiac, and its tributary, the left marginal, opened by a channel common to them and to the posterior veins of the left ventricle. The channel collecting these three veins was slightly dilated, possibly owing to the force of the blood coming from two directions and flowing together before emptying into the sinus. The oblique vein of Marshall, which normally empties into the coronary sinus from the left base of the heart, was absent. The narrow communication between sinus and auricle was connected with the former just superior to the opening of the middle and lesser cardiac veins.

The region of the right auricle which normally receives the coronary sinus was considerably dilated and its inner walls were lined by musculi pectinati. The valve of Thebesius was absent, and the opening of the small sinus connection was located with some difficulty. When examined from the inside this dilated portion of the auricle resembled an accessory auricular appendage.

Connected with the left innominate vein about 5½ cm. from the union of the two innominates to form the right superior vena cava, the left superior vena cava extended down over the arch of the descending aorta, and posterior to the heart. Penetrating the pericardium, the vessel coursed across the left auricular appendage and into the coronary sulcus, anterior to the left pulmonary veins. Its total length was 11 cm. It entered the coronary sinus at an angle, just superior to the opening for the great cardiac vein. Opening into this left superior vena cava were the accessory hemiazygos and the left superior phrenic veins.

The small size of the channel between the coronary sinus and the auricle indicates its probable inadequacy for taking care of any large part of the coronary blood. Most of it probably drained from the sinus by way of the anomalous vena cava, the left innominate, and thence to the right heart.

The figure which accompanies this paper is from a drawing by Miss Elizabeth Brödel, artist to the Duke University Hospital and Medical School.

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REGENERATION IN LIGATED OVARIES AND TRANSPLANTED OVARIAN FRAGMENTS OF THE WHITE RAT (MUS NORVEGICUS ALBINUS)

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TWO PLATES (TWELVE FIGURES)

INTRODUCTION

Several contributions on the regeneration of the ovary have appeared since Davenport ('25) reported that 64 per cent restoration occurred in the mouse after ovariectomy. Although many investigators have not recorded as high a percentage of recurrence as Davenport claimed in the mouse, they have found that there is a reappearance in some instances. I mention here only a few of the cases, notably 2.88 per cent in the rat (Heys, '31), 5 per cent in the mouse (Haterius, '28), and 9.1 per cent in the mouse after removal of ovary, capsule, and tube (Brambell, Fielding, and Parkes, '28).

According to most of the authors, regeneration is more liable to occur in older animals. The reason for this is thought to be that complete ovariectomy is more difficult in older individuals, and that pieces which hypertrophy are left behind. The determining factor of regeneration, therefore, is, indirectly, age. The possibility exists, however, that removing the ovary from an animal may inhibit the normal functioning of other factors which, in turn, would exert an influence on the regeneration of the ovary.

For the most part, investigators have been content to remove the ovaries and later study the extent of reappearance. Careful observations have not been made, so far as I am able to determine, on the nature of the regeneration of fragments and what is responsible for the material regenerated.

It is generally known that the remaining ovary will undergo tremendous hypertrophy in semi-ovariectomy. I have only to cite the works of Hatai ('15), Hartman ('25), and Solnacker ('27) to substantiate this statement. Hatai found in the rat that upon semispaying the remaining ovary increased 131.8 per cent in weight. Upon removal of one ovary and severance of the other from the tube there was 51.3 per cent increase. In the opossum, Hartman claims that the surviving ovary increased one and one-half to eight and one-half times. Solnacker records that the remaining ovary hypertrophies to twice its normal size when semi-ovariectomy occurs in the rat.

The hypertrophy in these experiments is said to consist of an increase in the number of corpora lutea and graafian follicles. More eggs must, therefore, mature to account for them. Since more eggs mature, possibly fewer follicles become atretic or more eggs are formed. The ovary might, on the other hand, lose its fecundity sooner. We have no evidence that the latter occurs. Part of this increase might result from the proliferation of more germ cells from the germinal epithelium such as the author ('27) found occurring in the ovaries of rats from six or seven days after birth until old age. Confirmatory and very excellent papers have since been published by Hargitt ('30, a, b, c).

After these various investigations, several problems still confront one. Among them are: 1) What is responsible for this new material, and what is the structure of these regenerated ovaries that have appeared? In other words, if a small piece is left behind, to what extent will it hypertrophy, what will constitute the regenerated mass, and from what does the new material originate? 2) What is the final fate of those ovaries which enlarge so greatly after semi-ovariectomy? If the remaining ovary contains so many mature graafian follicles and corpora lutea, it certainly seems that all of the oocytes would soon be used up or there would be increased activity of the germinal epithelium in forming more.

To investigate the above problems, two methods of attack were planned. In one set of experiments, the ovaries and upper ends of the oviducts were tightly ligated. It was hoped that this would suffice to kill the germ cells and cause degeneration within the ovary. If any regeneration occurred, it could be carefully followed. The other plan consisted of removing the ovaries and then transplanting a very small piece near the kidney. If no more germ cells are added from the epithelium, the small piece should soon lose its fecundity.

The purpose of this study, therefore, is to observe the tissue changes in ligated ovaries and transplanted ovarian fragments.

MATERIAL AND METHODS

Thirty animals were used in the ligation experiments. They ranged in age from forty to one hundred days. After removing the hair, a single dorsolumbar incision was made through the skin, and the ovary and tube were pulled through a lumbolateral incision. The ovary within the capsule was then tightly ligated with strong cord and returned to its former position. The wound was sutured with silk thread. Although there were few aseptic precautions taken, no infection resulted and healing took place rapidly. Very little mortality resulted from anesthesia. In all instances both ovaries were ligated. Beginning with the sixth day after the operation, ligated ovaries were collected from different individuals on successive days and were fixed in picro-aceto-formol. Thus the series consisted of ovaries from six to thirty-four days after the operation. Serial sections were mounted in all cases.

In the transplantation experiments, thirty rats, ranging in age from 25 to 150 days, were used and similar incisions were made as in the ligation operations. The capsule of both ovaries was split with iridectomy scissors and the ovary and stalk were removed. When there was the slightest reason to doubt complete ovariectomy the capsule was also removed. A very small piece from each ovary was then placed in the

fatty tissue surrounding the kidney. Pinching off blood vessels sufficed to stop most bleeding. The wounds were sutured, and the recovery was rapid in every case. All extirpated material was fixed in picro-aceto-formol, and was kept for subsequent microscopic examination. At varying intervals after the operation the rats were opened and the implanted pieces were removed, fixed, and serially sectioned. The series consisted of recovered ovaries from 9 to 157 days.

THE FATE OF THE LIGATED OVARIES

The ovaries of 90-day rats which had been ligated for eight or ten days consist almost entirely of degenerate tissue (fig. 1). Necrotic changes are particularly abundant in the central region, which consists of a connective-tissue framework and degenerating cells. The cells are enlarged, vacuolated, and contain pycnotic nuclei. The peripheral region often presents a slightly more normal histological appearance. However, in some areas the ovary and the capsule are inseparable and inflammatory lesions occur. Where an intervening space exists between capsule and ovary, the germinal epithelium seems to be normal. The peritoneal fluid may provide enough nourishment to enable it to survive.

All centrally located follicles are highly degenerate, and it is often extremely difficult to even find traces of them. In the periphery of the ovary, particularly in the region where a cavity has persisted between the gonad and capsule, one may encounter primary follicles. I have never found over twenty such follicles in an entire ovary and often they are completely lacking. Successive stages in the new formation of germ cells from the germinal epithelium give evidence that these follicles were derived from a postoperative proliferation of the germinal epithelium. More evidence for this assertion is found in ovaries which have been ligated for six days and contain no normal follicles.

The extent of recovery of vascular relations varies immensely in the various ovaries. The ovary is usually about one-third as large as when ligated. The degeneration of follicles and corpora lutea causes this shrinkage.

Figure 2 illustrates an area which is recovering in an ovary of a 90-day rat twelve days after ligation. Two normal primary follicles have recently differentiated from the germinal epithelium. A large cell with germ-cell characteristics has just been pinched off from the germinal epithelium. Adjacent to it is a similar enlarging cell. This is a frequent occurrence and is very interesting. The stroma has become quite normal.

The ovaries of rats sixteen days after ligation show increased signs of recovery. This recovery is not equally distributed over the entire ovary. Frequently it will be confined to one end of the ovary (fig. 3), while the other end remains in a moribund condition. The reason for a more rapid recovery of one end than the other is, no doubt, the more rapid restoration of the vascular supply, as may be seen from the figure. The stroma of this region appears more normal in structure and stains more perfectly. No double-layered and graafian follicles are found, and only an occasional primary. Sometimes the restored area has the form of a papilla (fig. 4), while the rest of the ovary remains very degenerate. Isolated primary follicles may be found in the tunica of such papillae.

Ovaries of animals twenty-one days after they have been ligated have often recovered considerably, depending upon the extent of their revascularization (fig. 5). Many of the follicle cells of atretic follicles have undergone fatty infiltration. Some of the regenerated portions contain only primary follicles. Others have recovered to the extent of having medium-sized follicles. Cells of an interstitial nature are very common in such ovaries.

Recovery is practically complete in ovaries after thirty to thirty-four days of ligation (fig. 6). Graafian follicles and secondary follicles are common. One also frequently finds a germ cell which has recently been proliferated from the epithelium.

In one rat the ovaries and the upper ends of the fallopian tubes were cut loose. The fallopian tube and the blood vessels

were then tied off. Thirty-two days later a small area in each ovary had recovered to the same extent as in those ligated for only ten to fifteen days. Each contained only two or three primary follicles and non-germinal epithelial cords.

The rate of recovery in the various ovaries after ligation differs considerably. Some even present a more normal appearance after twelve days than do others after sixteen to twenty days. About 50 per cent never recover, but are found as very degenerate bodies. The stroma undergoes marked signs of degeneration, yet it is capable of surviving if it receives a new vascular supply within a few days. It often becomes infiltrated with numerous lymphocytes, and its cells are extremely vacuolated. The germ cells are much less resistant, and degenerate if the vascular supply is impaired for very long. Few follicles of any description are found after six or eight days if the ovary has been completely ligated. It appears that ovaries of young animals (40 to 50 days) are slower in recuperating than ovaries of older animals. A gradual increase in the number and in the development of the follicles as time progresses after degeneration indicates that oocytes are added as a result of proliferation from the epithelium. Regeneration of the ovarian tissue, therefore, depends upon its revascularization and upon the activity of the germinal epithelium.

REGENERATION IN TRANSPLANTED OVARIAN FRAGMENTS

In these experiments, as previously stated, great precautions were used in obtaining complete ovariectomy. A small piece of the ovary was then transplanted into the fatty tissue next to the kidney. This place was chosen because it gave an environment which was similar to that of a normal ovary. Likewise, the ovary was far enough from the original site to be distinctly distinguished from any tissue which might reappear at the end of the tube. In one or two instances pieces were planted along the uterine horn, but far from the ostium so that no doubt would exist as to their identity. Seventy-two

per cent of all transplanted ovarian fragments were recovered. The rats were opened and autopsied. In most cases the piece could be readily found by the outstanding vascular conditions which had been newly created. Wherever it was doubtful, the material of that region was removed and later serially sectioned. Only one case showed the presence of ovarian tissue which had not been suspected on autopsy. Only two pieces of ovary which could not be accounted for were found at the end of the tube.

The particular rat which I wish to describe was 120 days old when the ovary was removed and a small piece transplanted. Nine days after the operation the piece in figure 7 was recovered below the kidney. The graft is encapsulated by connective tissue. The germinal epithelium cannot be identified, as this fibrous sheath is closely applied to the external surface of the graft. If it were not for the presence of the primary follicle, there would be little resemblance to an ovary. I have found two other such follicles in the same transplanted piece. Some sections also show a trace of luteal tissue. There are indications of a hilus in some sections. This figure well illustrates the approximate size of fragments that were transplanted in each case.

Large graafian follicles and double-layered follicles are common in ovarian fragments which have been allowed to remain for twenty-five days (fig. 8) in their transplanted position. The enormous increase in the size of the ovary has resulted principally from this enlargement of follicles. Some ovaries have hypertrophied to the extent that they contain ten to twelve graafian and medium-sized elements. Few primary stages are found, and it appears that most of the energy is expended in maturing some follicles rather than in increasing the number.

There was a great difference in the hypertrophy of two transplanted ovaries of the same rat. On one side the gonad resembled that shown in figure 8. On the other side the ovary was very small and consisted of degenerate follicles and a few lutein bodies. It is difficult to offer an explanation

as to why one ovary should be so greatly stimulated and the other somewhat suppressed. Perhaps the difference in the extent of vascular supply was the deciding factor.

Instead of finding so many large graafian follicles and double-layered follicles as in the twenty-five-day ovaries, one observes that by forty days they have been largely replaced by lutein bodies (fig. 9). Recently formed germ cells are seldom found. It seems that the few which are proliferated mature rapidly, thus leaving the ovary devoid of follicular stages. The graft is so closely encapsulated in many regions that it is difficult to identify a germinal epithelium in all instances.

In fifty-five-day transplants the follicles have been replaced almost entirely by corpora lutea atretica. Apparently degeneration overtakes the oocyte in many cases, while the stratum granulosum remains in an active condition. Thereafter the follicle cells undergo lypolytic degeneration. The presence and crowded condition of the numerous lutein bodies may retard or suppress proliferation of more germ cells and their further development.

Although the ovary one hudred days after transplantation is still filled with corpora lutea (fig. 10), it differs from that of fifty-five days in that it is much larger and a greater proportion of the lutein bodies are degenerating. As a result of the increase in size and marked regression of luteal tissue, the ovary has a slightly looser structure than formerly.

In the 125- to 150-day stage, one observes that the ovary is still larger, making room for the corpora lutea, for the proliferation of germ cells, and for the growth of the follicles (figs. 11 and 12). One can find all stages of proliferation from enlarging germinal epithelial cells to the primary follicle. The stroma is looser in structure and more vascular. It also seems more cellular. The regenerated ovary now presents characteristics of a typical normal ovary. During the regeneration two of the rats had litters which consisted of two and three individuals, respectively.

From the observations recorded, it is obvious that a small ovarian fragment can hypertrophy and become as large as the original ovary. Soon after transplantation most of the germ cells degenerate and they are then replenished from the germinal epithelium. Most of these develop and are replaced by corpora lutea atretica. When one of the lutein bodies degenerates, another follicle rapidly develops and undergoes a similar fate of fatty degeneration. For a long time, therefore, this small ovarian fragment consists almost entirely of lutein bodies. As it gradually increases in size, there is more room and we find both developing follicles and lutein bodies. By 125 days the ovary has increased in size to the extent that corpora lutea no longer fill it, and one is able to find different stages of follicle development and more germ cells which have formed from the epithelium.

Pieces of ovaries of rats 100 days old at the time of transplantation hypertrophy faster than do those from ovaries of animals 30 or 45 days old. One would think that the epithelium of a younger ovary would have greater potentialities for growth than an older, but this is evidently not the case.

SUMMARY

While the objective of many investigators has been to determine whether or not a new ovary can regenerate from tissue which has not previously existed as such, this has not been my purpose. I have only attempted to investigate the potentialities of the germinal epithelium and its regenerative capacity, and to strengthen my previous assertion that germ cells are formed from the germinal epithelium during the life of a rat.

The ligation experiments have sufficed to show that the germ cells have little resistance and are relatively short lived when the vascular relations are impaired. They have also shown that the germinal epithelium may survive and replenish the germ cells.

As soon as the ovary is revascularized, which takes from ten to twenty days, the germ cells which have been prolifer-

ated are able to continue their development. This explains the presence of new follicles. It is interesting that these observations closely correlate with Tamura's ('26). This investigator found that sixteen days after transplantation of ovaries in mice, a new proliferation of cells occurred which gave rise to young oocytes.

Among the many points brought out by the transplantation of the ovarian fragments are: 1) The extent to which a small fragment will regenerate; 2) that germ cells continue to be formed from the germinal epithelium; 3) the growth possibilities of the epithelium; 4) that the presence of too many corpora lutea inhibit germ-cell formation and the development of follicles; and, 5) that the enlargement of the fragment is at first due to many corpora lutea and graafian follicles. Upon the degeneration of many of the lutea and an increase in the size of the ovary, more germ cells are proliferated and follicles again become numerous.

The exact nature of how the corpora lutea inhibit germ-cell formation is not known. It may be that it is the pressure exerted by the lutein bodies within the ovary. In other words, the ovary is so completely filled with corpora lutea that there is no room and the germinal epithelium proliferates no cells inwardly. Growth mainly occurs peripherally. On the other hand, progestin may prevent it, since it probably inhibits mitoses in the endometrium (Burch, '31), ovulation, and the production of follicle hormone (Smith, '31).

It is equally interesting that a piece from an older ovary has greater and quicker regenerative capacity than a piece from a younger ovary. The question naturally arises whether or not the removal of the ovary in a young animal may inhibit the growth and maturity of some endocrine gland, which in turn may fail to stimulate follicular development and even regeneration of the ovary. We know that pituitary transplants will cause precocious gonad development (Smith, '27, b). The follicles, likewise, fail to develop upon hypophysectomy (Smith, '27, a). It is quite possible that the gonad-pituitary relationship may be disrupted on

the removal of an ovary to the extent that the pituitary of a young animal will not exert the same influence on regeneration as the pituitary of an old animal which had become mature. In other words, is the age factor the determining factor—that is, do ovaries regenerate in older animals because some fragment is left behind, or is some stimulating factor impaired by the removal of the gonad?

Parkes ('27) exposed the ovaries of mice to x-rays and found that all of the germ cells were killed. Cells were later proliferated by the germinal epithelium. None of these, however, became germ cells. In many instances they became like luteal tissue. He concluded that no new oocytes were formed after irradiation, and did not think that irradiation could have divested the ovary of the power of forming new oocytes during adult life. Therefore, he considers that no formation of new oocytes normally takes place in the adult ovary.

My experiments suffice to show that the epithelium can proliferate germ cells in the adult ovary. Whether the x-rays killed the germinal epithelium and divested it of its power to form germ cells, or whether the presence of scar tissue and luteal tissue prevented the formation of oocytes, one cannot say.

CONCLUSIONS

1. All of the germ cells in the rat's ovary rapidly degenerate after ligation of the gonad.

2. Upon revascularization, germ cells are proliferated from the germinal epithelium and are able to continue their growth and form follicles.

3. Usually by thirty-five days after ligation the ovary again resembles a normal ovary.

4. The enlargement of a transplanted ovarian fragment is first due to the formation of many corpora lutea and graafian follicles. After the degeneration of many of the lutea and an increase in the size of the ovarian fragment, more germ cells are proliferated and follicles again appear.

5. The germinal epithelium, therefore, has great growth capacity and germ-cell-forming properties, since it is capable of replenishing an entire ovary after degeneration of the germ cells, and can contribute so much to the hypertrophy of a small transplanted fragment.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Fixation for all sections was picro-aceto-formol; all sections were stained in hematoxylin and eosin. *E*, recently formed germ cells; *G*, germ cells being proliferated and enlarging.

1 Ovary from rat, ten days after ligation, to show the degenerative condition of the ovary. × 70.

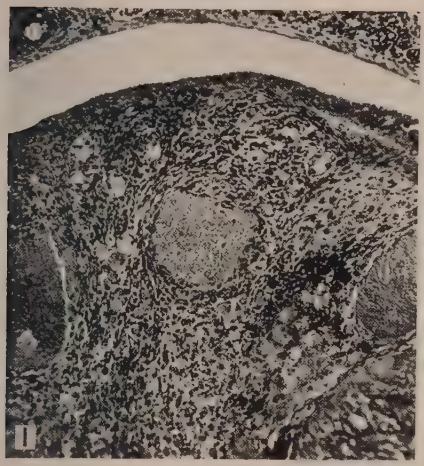
2 Photograph showing an area which is recovering in an ovary twelve days after ligation. Note the formation of germ cells. × 340.

3 Ovary from a rat, sixteen days after ligation, illustrating the uneven recovery as a result of the vascular relations. × 42.

4 This figure shows the new growth area in the form of a papilla. Note the recently formed germ cell. × 90.

5 Ovary from a rat, twenty-one days after ligation, showing the extent of recovery. × 95.

6 This figure shows that the ovary has reached a normal condition by thirty-four days after ligation. × 75.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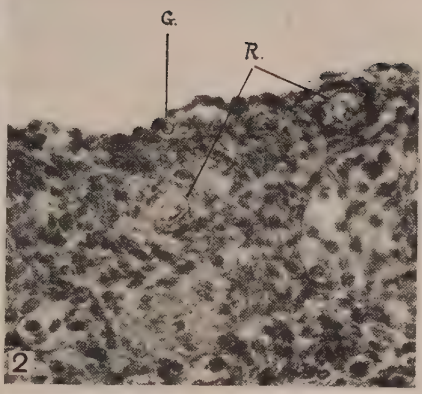
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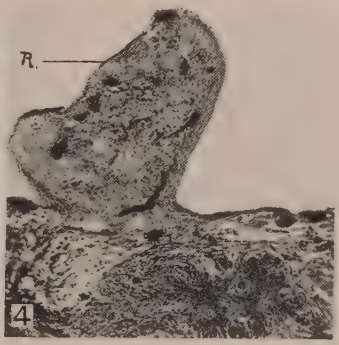
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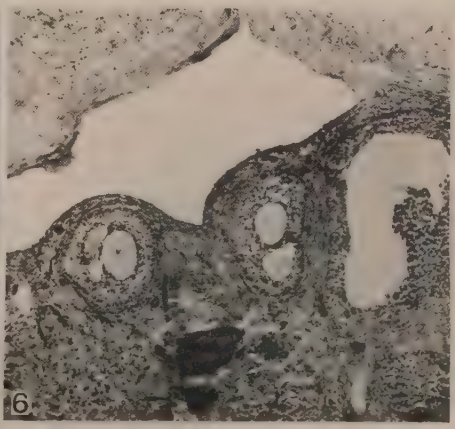
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PLATE 2

EXPLANATION OF FIGURES

P, primary follicle; *R*, recently proliferated germ cells.

7 Photograph showing a small transplanted piece after nine days. × 244.

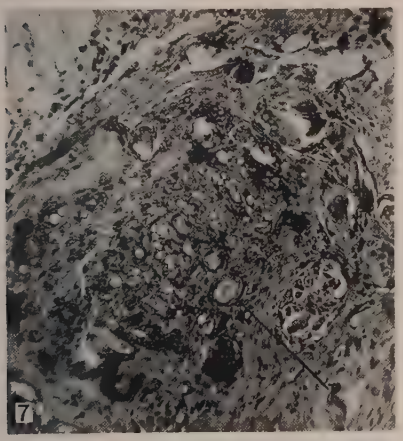
8 This figure illustrates the growth of follicles in a fragment which had been transplanted twenty-five days previously. × 72.

9 Ovary resulting from a piece transplanted forty days previously. Note that lutein bodies have replaced the follicles. × 28.

10 This figure shows that the ovary has enlarged and has become somewhat looser in structure, although mainly lutein bodies fill it. × 56.

11 This figure illustrates in an ovary 150 days after the operation a new growth area which is devoid of lutein bodies and contains numerous recently proliferated germ cells. × 40.

12 Photograph illustrating the normal condition reached by the transplanted ovarian fragment 125 days after transplantation. × 44.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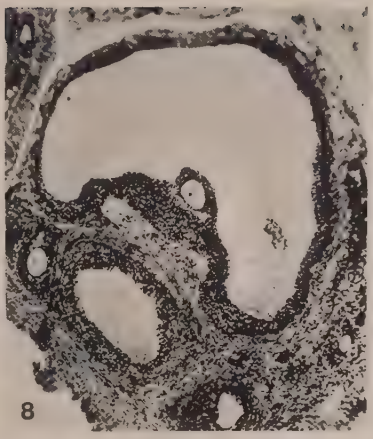
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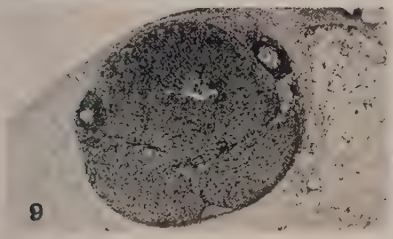
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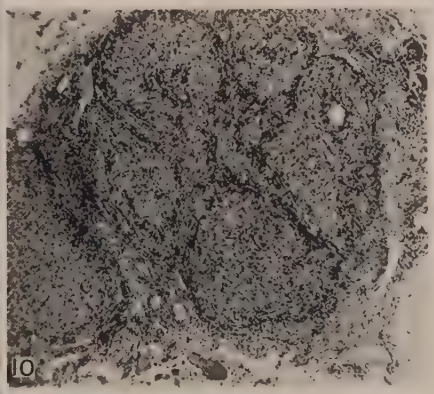
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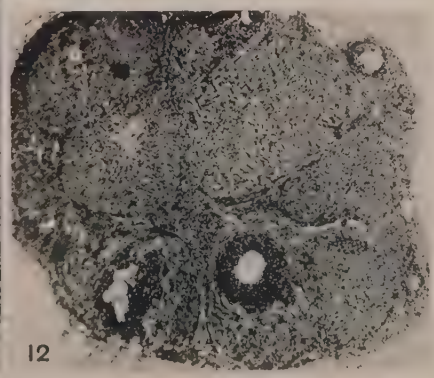
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CONTRACTION OF BLOOD VESSELS AND OBSERVATIONS ON THE CIRCULATION IN THE TRANSPARENT CHAMBER IN THE RABBIT'S EAR

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SEVEN FIGURES

The contractility of small blood vessels is a subject which has been studied in the living by a number of investigators during the past sixty years. Before Stricker ('65) saw the phenomenon of independent contraction of capillaries in the nictitating membrane of the frog, these vessels were thought to respond to circulatory changes in a passive manner only, the flow of blood through them being regulated entirely by the contraction and dilatation of vessels surrounded by muscle cells. Lister ('58) had noticed a dilatation of capillaries in inflammation. The further studies of Golubew ('69), Rouget ('73), Tarchanoff ('74), Roy and Brown ('79), Steinach and Kahn ('03), and many others, established the fact that capillaries are contractile as well as elastic. Most of these observers studied the capillaries of amphibians and obtained contractions by using mechanical, electrical, or chemical stimuli.

Renewed interest in capillary contractility has recently been aroused by the studies of Vimtrup ('23), working under Krogh ('22). Vimtrup made a careful study of the adventitial cells of capillaries which were first described by Rouget, and which he termed 'Rouget' cells. Using the transparent tails of living amblystoma larvae, he observed definite contraction of capillaries without any special stimulus, and

described it as being inaugurated by the contraction of Rouget cells. Parker ('23) and Federighi ('28) described, in certain invertebrates, the active contraction of endothelium of capillaries on which no adventitial cells could be seen. E. R. and E. L. Clark ('25), repeating Vimtrup's studies on larvae of Amblystoma and of Anurans, found active contraction of capillary endothelium, but failed to corroborate Vimtrup as regards the part played by the 'Rouget' cells, since they observed contraction of capillaries on which there were no 'Rouget' cells, and since, in capillaries on which sparsely distributed 'Rouget' cells were present, the inauguration of the contraction occurred much more often away from the 'Rouget' cells than in their immediate neighborhood. Moreover, they noted that the endothelium contracted away from the 'Rouget' cell, leaving a clear space between.

It is, then, clearly established that in Amphibia capillary endothelium possesses the property of active contractility, and that capillary contraction plays an important rôle in regulating blood flow.

For the mammal, until recently, nothing has been definitely known, in spite of a large number of studies by Hooker, Krogh, Rich, Hill, Florey and Carleton, and others. The reason for this uncertainty has been the unavailability of any region in a mammal in which the smallest blood vessels could be seen with sufficient clearness in the living under normal conditions. This need has been supplied by the artificial production of a tissue so thin and transparent that the individual cells of the walls of arteries, veins, and capillaries may be seen clearly with the highest microscopic lenses, and in which prolonged studies may be made of the same vessel similar to those carried out in the tail fins of amphibian larvae. The method developed in this laboratory, under the direction of Dr. Eliot R. Clark, consists of the introduction into a hole made in the ear of the rabbit of a thin, transparent, double-walled chamber with its sides open to the deeper tissues of the ear, and into which new blood vessels and connective tissue grow, forming a tissue which is per-

manent and which is essentially similar to the subcutaneous tissue of the ear (Sandison, '24, '28; Clark, Kirby-Smith, Rex, and Williams, '30). As described in a previous paper (Sandison, '28), the newly formed blood vessels undergo a complete differentiation into arteries, arterioles, capillaries, and veins. Recently (Clark et al., '31), a description has been given of the ingrowth and differentiation of blood vessels in sixty standard chambers under controlled and approximately uniform conditions, with a correlated study of the circulation, and the subsequent changes in the vascular pattern over a period of months (over a year in several cases).

The studies to be described here were made on chambers constructed entirely of kodaloid (Sandison, '28). The vessels were all new ones which had formed by ingrowth into the chamber from the subcutaneous vessels of the ear, and the observations were carried out largely on a chamber in which the vessels were from two to four and one-half months old. The major purpose of the study was to find out, if possible, just what elements of the minute vessels are responsible for the regulation of flow through the capillaries, whether the capillary endothelium is contractile, what part the adventitial or 'Rouget' cells may play, to what extent the flow is regulated by the smooth-muscle cells, and what other factors may be concerned with the capillary circulation. It should be mentioned that nerves were not seen in the present observations on the new vessels, and it was uncertain whether they were present or not. However, important as this question may be from many standpoints, it does not seriously affect the primary object of this study. A preliminary account of these observations was presented before the Physiological Society of Philadelphia, May, 1928.

OBSERVATIONS ON CONTRACTION

It was early noted that, in a well-developed plexus, there were frequent changes in the circulation; periods of active flow alternating with periods of marked slowing or even of

actual stasis. Careful study showed that the circulation in any given plexus of vessels changes in an almost rhythmical manner.[1] On the average, about twice in each minute, the free, active flow of blood is interrupted by a slowing or stasis of a few seconds duration. Examination of the various parts of the system to discover the factors responsible for this periodic slowing brought out certain interesting facts. It became clear that it is the contraction and relaxation of the smooth-muscle cells of the arteries and arterioles which cause the changes in the circulation. The adventitial or 'Rouget' cells, which are present in abundance on the precapillaries, play no part whatsoever in the contraction, and the endothelium of the capillaries displays a power of contractility so slight that it is not a factor in the regulation of the blood flow.

The contraction sometimes appears first in the main artery of the ear, which narrows markedly, and may extend as a wave along the arteries and their branches and the arterioles until the last smooth-muscle cell is reached, three or four seconds being taken up in the passage of the wave from large artery to small arteriole. The narrowing in the different parts of the arteries may be partial or it may be sufficient to block completely the flow of blood. Contractions of the smooth-muscle cells around the arterioles do not occur simultaneously in all arterioles, and as a result of this alternating contraction, the blood may flow first in one and then in the opposite direction in the same vessel (fig. 1). The smooth-muscle cells on the larger arteries may remain contracted for long periods of time under certain conditions, while the mus-

[1] Clark and Clark ('32) have made observations on the living preformed blood vessels, using the 'preformed tissue' chamber (Clark et al., '30), in which the main artery of the ear is retained and a thin, transparent area about 1.5 cm. in diameter, including the preformed arteries, veins, and capillaries. They describe the normal occurrence of spontaneous rhythmic contractions of arteries, involving the main artery of the ear, down to smaller and smaller branches, and find that the different arteries and parts of the same artery each contract at a different tempo and that the periodic contractions have a profound influence on the distribution of blood to different areas and on the direction of flow in different vessels.

cle cells on the arterioles rarely remain contracted for more than a few seconds.

That it is the smooth-muscle cell which is almost exclusively responsible for the contraction of vessels comes out most strikingly from a study of the smaller arterioles and capillaries. In the arterioles the continuous sheet of smooth-muscle cells is succeeded by muscle cells in groups, and these in turn by pairs, and finally by isolated single cells. They can be readily seen, especially with the oil-immersion lens, as small round structures outside of and encircling the endothelium. They can be distinguished easily from the adventitial cells which lie longitudinally along the vessel, and which

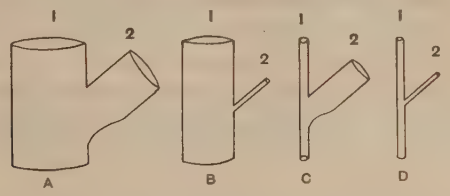

Fig. 1 Camera-lucida tracings, showing the size of the lumen of an artery (1) and its branch (2), in different phases of dilatation and contraction. A, both 1 and 2 dilated; B, 1 partially, 2 completely contracted; C, 1 completely, 2 partially contracted; D, both 1 and 2 completely contracted. × 310.

are larger in cross section. If such an area is watched it will be seen that the arterioles contract at the places where the smooth-muscle cells are present, and that the vessels beyond the last cell narrow only to a very slight extent, if at all.

It is most fascinating to watch the contraction of a single isolated smooth-muscle cell on an arteriole (fig. 2). When undergoing a contraction, it causes a rapid narrowing so that, in the course of two or three seconds, the lumen may be so constricted that no blood cell can pass. It remains contracted for three or four seconds and rather suddenly relaxes, the relaxation being immediately followed by a rush of blood through the vessel. It remains relaxed for periods of time varying from ten seconds to a minute, and then contracts

again. The smooth-muscle cell does not always constrict the lumen completely, for it may contract only partially and narrow the lumen without obliterating it. The periods of relaxation are definitely longer after the partial contraction.

While these definite contractions are occurring in the smooth-muscle cells, which regulate the flow of blood, there are only such general changes in the capillaries as can clearly be explained on the basis of elasticity and of outside or inside pressure. Following the shutting off of the blood flow, there may be a slight general narrowing of the capillary, to be followed by a slight widening when active circulation is re-

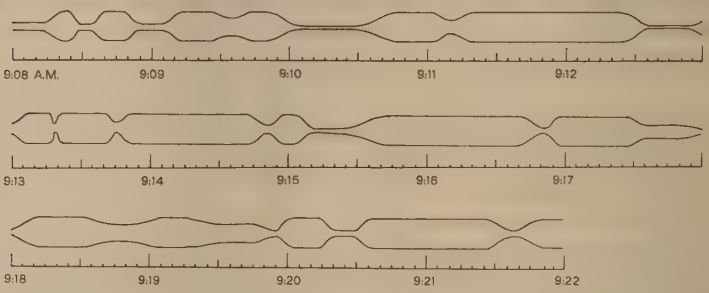

Fig. 2 Diagram showing the degree and rate of changes in caliber of an arteriole following contraction of a single muscle cell which almost completely surrounded the arteriole. The contraction periods of this cell occurred at the same time as did the rhythmical ones of the arteries within the whole chamber.

sumed. But these changes are obviously secondary to the contraction and relaxation of the smooth-muscle cell on the arteriole. At times there has been observed a complete emptying of an extensive capillary plexus, coincident with a reduction in blood flow, and occurring simultaneously with the contraction of the large supplying artery. This simulated capillary contraction, but was found to be due to the pressure exerted on the tissue by the thin, elastic celluloid cover of the chamber, for pressure over the area with a fine glass rod produced an identical picture. Evidently the widening of the arteries raises the cover slightly, permitting easy circulation through the capillaries, while the contraction of arteries

allows the cover to spring back and compress the capillaries, particularly over the tables. This was further proved by constructing chambers in which the covering celluloid was cemented in places to the tables to make it rigid. In such chambers the capillaries did not show this peculiar type of emptying.

The circulation is markedly influenced by the development of muscle cells on the newly formed capillaries as they become transformed into arterioles. Changes in caliber were observed in the same vessels both before and after the appearance of muscle cells on their walls. It was found that before the development of these cells the narrowing of such vessels was always very slight, involved the whole vessel uniformly, and followed the rhythmic contraction of the main artery just described. After the appearance of muscle cells, however, the same vessels were seen to display localized narrowings, and such constrictions always occurred in the region of the newly developed smooth-muscle cells.

OBSERVATIONS ON THE CIRCULATION

The manner in which blood flows through vessels and the behavior of the blood cells in the normal blood stream were observed and described many years ago by numerous investigators and only a few new observations have been made on the circulation as studied in the rabbit's ear. However, since most of the previous work on this subject has been carried out on the mesentery of mammals where one is limited to short observation periods, it is of interest to restudy the problem in the transparent chamber, where the same blood vessels may be seen from day to day for months, and the circulatory changes in them observed during both their early formation and their subsequent adult growth.

The different rates of flow in various kinds of vessels at different stages of growth, together with the causes for these variations, have already been discussed and partly analyzed (Sandison, '28). One sees in the blood current in the rabbit's ear the well-known axial stream of cells, and the compara-

tively narrow, clear, plasma layer, or 'randzone,' which surrounds this rapidly moving central core. The throwing off of the leukocytes into the peripheral layer and their slow rolling motion along the vessel wall is also a phenomenon which is readily seen.

In this study it has been observed that the presence of the clear peripheral stream is dependent upon the rate of flow: it is widest when the blood is flowing rapidly, and occurs chiefly in the arteries and arterioles, except when these vessels are so constricted that the single blood cells are squeezed in passing through. When the circulation slows down or stops, during a period of contraction of the arteries, this peripheral layer is absent in all vessels. At such times the erythrocytes and leukocytes are no longer limited to the axial stream, but wander at random; or they may settle to the dependent part of the lumen, leaving only a clear layer of plasma above. During a period of stasis, which may last for several seconds or minutes, this sedimentation of blood cells may be quite noticeable, particularly in the larger vessels.

Then, too, an uneven mixture of cells with plasma has been observed during a period of sluggish or irregular flow of blood through the capillaries—a condition which results in part from an irregular contraction of first one arteriole and then another. This independent contraction of arterioles (i.e., one vessel closed at the same time another is open) causes blood to be fed to the veins through the capillaries and venules in the form of a broken stream. An uneven mixture may also occur within an arteriole or an artery itself as a result of contractions occurring at intervals along their walls, leaving dilated portions of the vessel in which clumps of cells collect (*Art. G,* fig. 5). When such contracted regions again dilate and the circulation is resumed, the clumps of cells pass on without mixing evenly again until they reach the larger lumen of the venules. In this connection the work of Landis ('26) is of interest. He has been able to measure capillary pressures during capillary stasis in the frog's mesentery and to watch the flow of plasma through the endo-

thelium as indicated by the passage of injected dye. He finds that wherever such a flow is present there is a considerable change in the proportion of blood cells and plasma within the lumen.

The phenomenon of 'plasma skimming,' a term introduced by Krogh ('22), and that of leukocyte skimming may be exhibited in various ways. The former is seen mainly in partially contracted vessels or in capillaries which are cross connections between the main path of the circulation and which, even though dilated, have no circulation on account of equal pressures at their two ends; it may be seen also in blind-ending, new-growing tips. Not infrequently vessels containing skimmed plasma, i.e., plasma without erythrocytes, are loaded with leukocytes which, along with the plasma, have been skimmed off from the main circulation. In fact, leukocyte skimming may be seen in practically any uncontracted vessel in which the circulation has temporarily ceased, but which remains connected with other circulating vessels.

The following observation on platelet skimming was also made: in a single, very narrow capillary loop, whose two ends were connected to a large circulating vessel, plasma flowed at all times, as was indicated by the passing of the smallest blood platelets. For many minutes only platelets would pass. It was interesting to note that with an increase of blood supply through the large vessel, erythrocytes would be forced through the narrow loop. With a very rapid rate in the larger vessel, accompanied supposedly by an increase in blood pressure, even the largest leukocytes were forced through the capillary, although its entrance was so constricted that the cells, in passing, were forced out into very long and narrow forms.

Plasma skimming is not confined to capillaries, since it has been seen in arterioles which are constricted almost completely at their proximal end and dilated along the remainder of their course, the dilated portion containing only a single blood cell here and there. Such plasma skimming in an arte-

riole is a rather complicated condition, and certain factors must be present for its occurrence. In the first place, the flow of blood through any arteriole is dependent upon: the degree of dilatation and the rate of blood flow in the artery which supplies it, the pressures in the capillary bed which the arteriole itself supplies, the size of its entrance, and the caliber of its entire lumen. Plasma alone will enter the arteriole when its entire lumen or merely the lumen at its entrance, is constricted to a size much smaller than the blood cells, and when the pressure of its arterial supply is not sufficient to force cells through the constricted opening. When, as indicated by the passage of platelets, plasma flows through a vessel under these conditions, an occasional cell may enter it, but if both the pressure in the capillary plexus beyond it and that at its entrance become equalized (a condition which may result from the passage of blood through another arteriole into the same plexus of capillaries) blood flow will cease in this vessel, the cells will be drawn off, and true plasma skimming will usually follow. Krogh ('22), in studying the frog's web and tongue, saw a washing away of the erythrocytes, when he stimulated a portion of a small artery branching from a larger vessel almost to the point of complete contraction. He stated that the current of blood through the small artery seemed to cease altogether, and that at the same time the erythrocytes were washed out from the corresponding capillaries. In the rabbit's ear one also sees that the cells are washed away from a capillary bed, following contraction of the arterioles or arteries, but there still may be a continuation of the flow of the plasma containing platelets only.

In regard to the capillaries, however, this same plasma skimming may occur as the result of another factor, i.e., the reversal of flow in an arteriole—a phenomenon which occasionally takes place during a short period of stasis in the artery. In this case, the blood cells are drained out of the capillary plexus in the absence of circulation. It will be noted, therefore, that two types of plasma skimming have been observed in the vessels of the rabbit's ear, one in which

the vessels contain a circulating plasma and the other in which there is no circulation.

Venous and capillary pulsation have been seen in one plexus or another at all times during the new growth of vessels, particularly in vessels which are either short circuits between those which carry the main flow of blood, or blind-ending tubes. A very slight pulse is usually present in all young capillaries, but it is barely perceptible to the eye except when the rate of flow diminishes. In the older tissue, however, a well-defined capillary pulsation is extremely rare, except when the rate of flow is very rapid or very slow, and even then it is present in only a few vessels. One small plexus of blood vessels, containing a single pulsating capillary, was studied carefully in order to determine the cause of this pulsation. The plexus contained two capillaries which emptied close together into a very large vein, in which the flow was even and constant. When the circulation throughout the plexus became slow, one of these capillaries pulsated while the other did not. It was seen that these two capillaries were each supplied by a different arteriole, one of which supplied several other capillaries with blood, while the second arteriole had no other capillary branchings. In the latter arteriole the rate of flow was diminished, and it was in the capillary connected with it that the pulsation was observed. Blood cells were seen to enter the large vein from this capillary in an interrupted stream, a few cells with each beat of the heart. The flow through the entire vein was perfectly steady and it seemed probable that the pulsation in this particular case was present in the one capillary on account of the short connection between the arteriole and vein, and absent in the other on account of the greater number of capillaries which its arteriole supplied.

As for the venous pulse, it may be due to a transmission of the arterial pulse to the veins, or possibly to the contractions of the right auricle. It is difficult to explain the general pulsation which may occur in veins and capillaries when the circulation is extremely fast, as, for example, when the ear

is heated to 38°C. or above. At such a time the arterial wall is distended slightly at each beat of the heart, the arterioles are distinctly narrowed, the capillaries and venules are slightly narrow, and the rate of blood flow in the large veins is almost equal to that in the artery.

While the circulation in a capillary bed is almost entirely regulated by the action of muscle cells on arterial vessels supplying it, it has been found that the flow may be diminished or even stopped by a single leukocyte plugging a narrow vessel. This occurs frequently, but is of minor importance, since it is always temporary. One of the most favorable places for such plugging is at the origin of the small arterioles

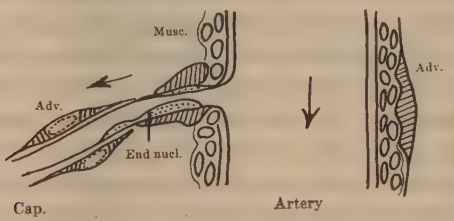

Fig. 3 Camera-lucida drawing of a precapillary branch of an artery. The artery has a double layer of muscle cells (*Musc.*); the entrance to the precapillary is almost obliterated by the bulging endothelial nucleus (*End.Nucl.*). Adventitial cell (*Adv.*); capillary (*Cap.*). × 412.

from their arteries. Normally, this region is constricted partly on account of the bulging of endothelial cells into the lumen. Such a bulging in a capillary is shown in figure 3. At times the constriction is too great to permit the passage of the leukocytes, though the more elastic erythrocytes may pass quite readily. Unless the force of the blood stream (always variable) is sufficient to push the obstructing cell onward, the latter may stop the circulation in the capillaries fed by the arteriole for varying lengths of time—sometimes for several minutes. One such arteriole was watched and the following was observed: With a rapid rate of flow, a condition which was always caused by a dilatation of the artery, both the erythrocytes and the leukocytes were forced through

the narrow lumen in the form of an unbroken stream, even though they were enormously distorted in passing. With a moderate rate of flow the erythrocytes passed through easily, though again distorted, but the leukocytes went through the constriction in a jerking manner, apparently due to the effect of the systolic waves upon them, and each one plugged the vessel for a certain length of time. Occasionally the leukocytes' own ameboid activity would assist their passage. The time required for the passage of a leukocyte through such a constriction depended upon the activity (blood pressure) of the general circulation, being greater with a feeble blood stream. At periods of a steady, rapid rate of flow, the number of leukocytes which passed through this arteriole was an average of forty per minute.

REACTION OF BLOOD VESSELS TO TEMPERATURE CHANGES

In addition to the factors just described, which change the rate of flow and the amount of blood distributed through the vascular system, one other phenomenon has been studied in this connection, namely, the reaction of vessels to temperature changes (figs. 4 and 5). The higher temperatures were produced by an ordinary 60-watt electric-light bulb, the degree of heat being regulated by the distance of the bulb from the ear. The temperature was measured by placing a thermometer a few millimeters above that part of the ear which received the greatest amount of heat. The vessels were studied at three different temperatures: 37°C., 26° to 32°C., and 20°C. (produced by placing sponges dipped in ice water directly on the surface of the chamber).

While the heat was being applied, the circulation gradually became more regular and faster, until, by the time the maximum of 37° was reached, it was quite regular in rate of flow and the speed was exceedingly rapid, being almost as fast in the veins as in the arteries. In addition to the rapidity of flow, a general pulsation of the vessels could be seen. The rhythmical contractions previously described now occurred only at very long intervals and lasted for a very brief period

of time. After the light bulb was removed and as soon as the ear became accustomed once more to room temperature, the circulation continued as it did previous to the experiment. The sticking of leukocytes to the walls of capillaries and their emigration through the vessel walls, following heating of the ear to 37°C., has already been described (Sandison, '31).

When the ice packs were applied, the branches of the larger arteries contracted completely, and all circulation in the chamber stopped. Very occasionally the arteries would open slightly and permit a sluggish flow of blood, but this partial dilatation had very little effect upon the capillary circulation. The exact point on the arteries where this marked contraction ended varied in all of them, but it rarely extended to the arterioles, most of which were slightly dilated. When the ear was brought back to room temperature, the circulation again returned to its normal condition.

The specific reaction of the different types of vessels during these experiments may be followed in figures 4 and 5. Here it is seen that the main vessels concerned in the regulation of the blood flow are the artery and arteriole. At 20°C., the artery, by its contraction, is alone responsible for the stasis which develops, the arterioles are incompletely dilated in the region of their muscle cells, while all other vessels are wider than at any other temperature. The rhythmic contraction of the arteriole disappears. The small vessels at 32°C. are on the whole narrower than at 20°C., and the rhythmic contraction of the arterial vessels is more regular at this temperature. At 37°C., the capillaries, the arterioles, and the arteries are smaller than at any other temperature; the veins, though larger than at 32°C., are smaller than at 20°C.

Fig. 4 Camera-lucida drawings, showing changes in the caliber of all types of vessels with three different degrees of temperature. The insert at the upper right corner shows the parts of the plexus from which the segments of different vessels shown in figures 4 and 5 were taken. *A*, arteriole; (*H*, origin of arteriole *A*); *B*, a precapillary; *C*, a loop of the prepostcapillary; *D*, a venule; *Dil.*, greatest degree of dilatation; *Con.*, greatest degree of contraction; *No Con.*, no contraction; *a* and *a1*, same group of muscle cells; *b* and *b1*, the same adventitial cell. × 310.

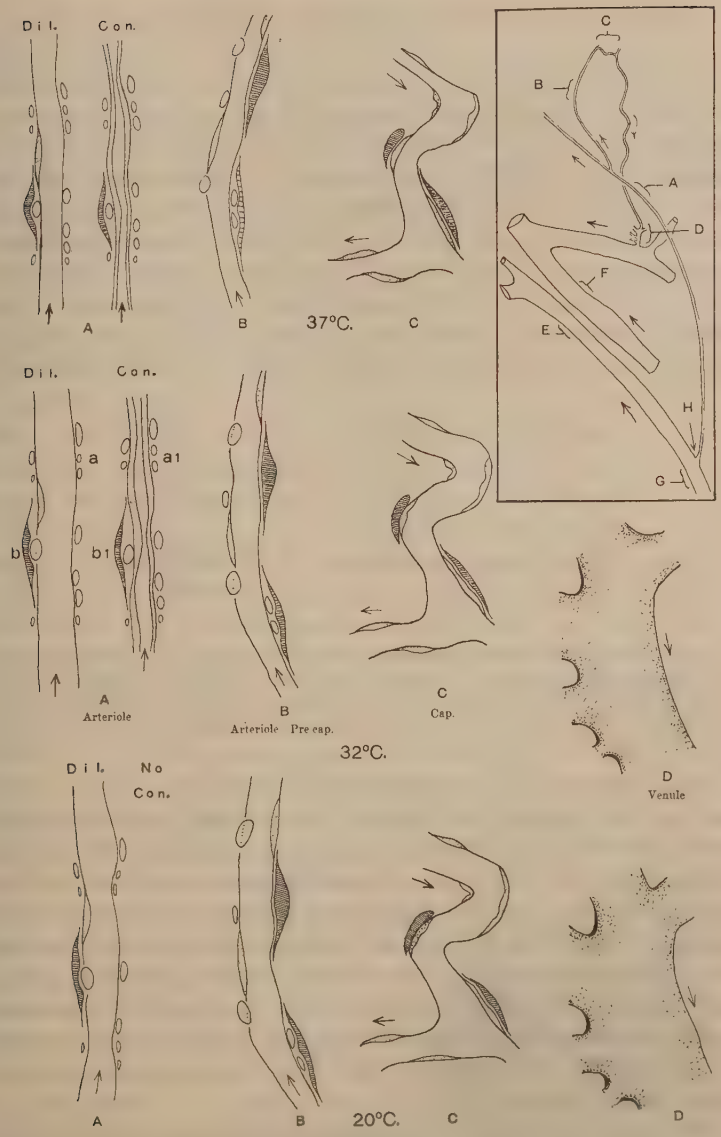
Dil.
Con.
A
B
37°C.
C
C
B
A
D
F
E
H
G
Dil.
Con.
a
a1
b
b1
A
Arteriole
B
Arteriole Pre cap.
C
Cap.
32°C.
D
Venule
Dil.
No
Con.
A
B
20°C.
C
D

Figure 4

REACTION OF THE BLOOD VESSELS TO ADRENALINE AND HISTAMINE

For these experiments a preparation was used containing adrenaline, 9/20 grain; chloretone, 2¼ grains, and physiological salt solution, 1 fluid ounce. For intravenous injection a dilution of 1:100,000 was employed. Observations of the blood vessels in the transparent chamber were made during the injection of the drug into a vein of the opposite ear, and a region among these vessels was selected where an artery of a caliber of over 125 μ, with its arterioles, capillaries, venules, and veins could be watched. This artery had at least three layers of muscle cells around it. Before the injec-

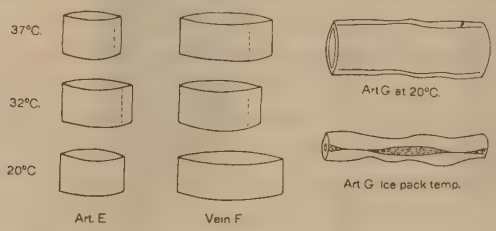

Fig. 5 Camera-lucida sketch, showing changes in caliber at different temperatures of three other vessels of the same plexus shown in the inset figure 4. *E*, an artery having two layers of muscle; *F*, a vein; *G*, the more proximal portion of the artery; *E*, point beyond which contraction did not occur at 20°. × 206.

tion was begun, the normal changes in caliber of this vessel and of its arteriole branchings were noted, and they conformed to the changes which have been previously described. At no time did this artery constrict more than one-half of its fully dilated caliber and the rhythm of its contraction was similar to that which has also been previously described for arteries in this chamber. Surrounding the wall of the proximal half of the visible portion of this large artery were numerous macrophages which contained a dark yellow-brown pigment. The vessel had slight local contractions here and there along its wall.

The injection needle was quickly thrust into the vein with one movement. Almost immediately the arterial vessels con-

stricted slightly more than normal, and in a few seconds they dilated fully. This is the usual response to any disturbance to the animal. Then, after one minute, at the end of which time these vessels were still dilated, a slow injection of the adrenaline was begun. The large artery began constricting one and one-half minutes after 0.3 cc. of the 1:100,000 solution had entered the vein. Injection was then stopped. A few seconds later the large artery constricted to about 25 μ in caliber (one-sixth its dilated caliber) along its proximal half. The degree of constriction was less along the remainder of its course, being less marked the more peripherally one followed it. The normal, locally contracted regions were the points of greatest constriction in any one segment of the vessel. The lumen, even though greatly constricted by contraction of the muscle cells which were much thicker than normal, widened slightly with each beat of the heart.

The arterioles were greatly constricted along most of their course, but they showed only a partial contraction near their capillary terminations, even though they were surrounded in that region by a single layer of muscle cells (fig. 6). They were most constricted at the point where they took origin from the large artery—a point where they are normally narrower (fig. 3). The venules also narrowed, but to a slight extent only. The capillaries were apparently unaffected, except for the usual very slight changes which normally accompany changes in blood pressure, i.e., a slight dilatation with decreased rate of blood flow. The flow of blood in the capillary bed varied in different capillaries from a complete stop to a moderate rate, depending upon their proximity to an arteriole. In most of the main pathways which lie between the arteriole and the venule a slow circulation prevailed.

One interesting observation during the contraction of the large artery was the effect upon the pigmented macrophages which surrounded a part of this vessel. They were apparently unchanged in regard to their position—a wide clear space between them and the muscle cells of the vessel wall being quite evident, whereas previous to the time of injection they had lain directly upon these muscle cells.

The constriction period in all vessels was not over two minutes, and one and two-thirds minutes after the injection was stopped the arterial vessels began to dilate; twenty seconds later, the dilatation was almost complete. At this moment a second injection was begun, the needle having been kept in place, and 1.2 cc. of the adrenaline solution was steadily emptied into the vein over a period of four minutes.

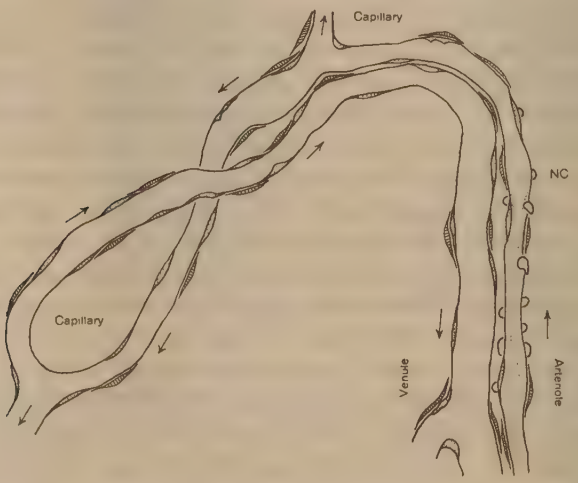

Fig. 6 Camera-lucida drawing, showing effect of an intravenous injection of adrenaline chloride (1: 100,000). The arteriole contracts, while the capillaries and venules are unaffected. Dotted line indicates degree and limit of contraction. Endothelial nuclei dotted, adventitial cells cross-hatched, smooth-muscle clear ovals. *N.C.*, no contraction beyond this point. × 316.

One and one-half minutes from the beginning of this second injection, after 0.3 cc. had again entered the circulation, there was a repetition of the change in caliber of the vessels similar to that noted after the first injection. At the end of two minutes, dilatation was not complete, but there was a considerable widening of the arterial vessels. The injection was continued, and one-half minute following this incomplete dilatation a constriction of the vessels again occurred, but to a much less degree than the one which just preceded it. The

vessels remained in this state of partial constriction for one and one-half minutes after the injection, at which time they dilated and this dilatation, which was general, lasted at least one-half hour. The effect of this arterial dilatation on the capillary circulation was marked and brought about a great increase in the rate and amount of flow through all vessels with the establishment of circulation in capillaries which were previously open but non-circulating.

Under the action of a more dilute solution of adrenaline (0.3 cc. of 1:500,000) the capillaries gave the same response, but there was less of the primary slowing of the capillary circulation, partly on account of a lesser contraction of the arterial vessels. When 2 minims of 1:1000 solution were injected subcutaneously, the vessels responded as they did with the intravenous injection, except that the contraction of arteries and arterioles was prolonged for seven minutes or more, and the return of the normal rhythm of these vessels appeared much later. Local application gives such a picture as shown in figure 7.

The effect of histamine upon the blood vessels was also observed. The same method of study as that used with adrenaline was employed, except that only one dilution (1:100,000) was used, and only the intravenous route of injection was chosen. The action of 0.004 mg. of this drug resulted in dilatation of arterioles and arteries with almost complete stasis throughout the vessels of the chamber for over two hours. The greatest degree of dilatation occurred in the arterioles, since they widened to twice their usual diameter. The capillaries were unchanged in caliber, except for the usual slight dilatation following lack of flow through them, and all of the previously non-circulating, wide-open capillaries were filled almost to capacity with blood in stasis. The very narrow capillaries, which are the retracting or the new-growing vessels, were observed particularly for dilatation, but they also were unchanged. Frequent reversals of flow in all vessels were noticed. The next day, eighteen hours later, $\frac{1}{2}$ cc. of adrenaline was administered subcutaneously in

the abdominal region, and all of the arterial vessels, which had dilated with the injection of histamine, then contracted.

The results, therefore, of the action of these two drugs on the vessels of the rabbit's ear show that the various responses are chiefly confined to the vessels which are surrounded with muscle cells. With dilute intraveous injections, adrenaline causes a fleeting constriction of all arterial vessels in the

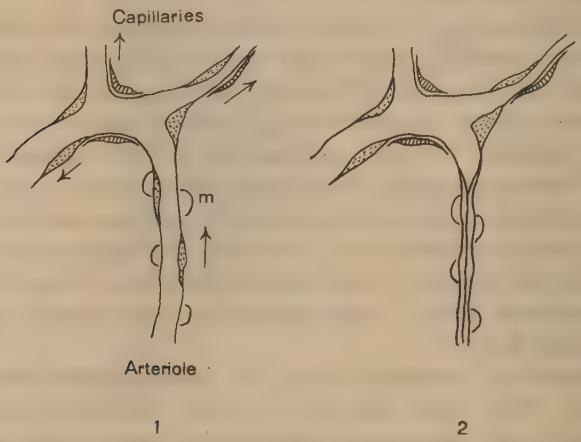

Fig. 7 Camera-lucida drawing, showing degree and limit of action of a local injection of 1: 1000 adrenaline chloride. *M*, muscle cell. *1*, preceding, and *2*, following injection. × 310.

chamber with diminished capillary circulation, which action is quickly followed by an arterial dilatation, accompanied by a much more rapid capillary flow than that usually present in the normal circulation. Histamine causes a still greater diminution of the capillary circulation, and dilatation of all the peripheral arteries and arterioles with the strength of solution used. Neither drug acts, apparently, on naked endothelium.

SUMMARY

Microscopic observations have been made on new blood vessels which have grown into a transparent chamber introduced into the rabbit's ear, and which have persisted for from two to four and one-half months, with the following results:

The local control of blood flow in this newly formed tissue appears to reside in the smooth-muscle cell on the arteriole. Adventitial ('Rouget') cells are present in large numbers, but do not appear to contract.

Capillaries proper, with or without perithelial ('Rouget') cells, have such a limited power of contractility that they seem to play no significant part in the control of circulation. While their caliber manifests changes, the changes seem to be passive rather than active, and brought about chiefly by changes in pressure both within and without, coupled with a certain degree of elasticity of endothelium. However, in the persistent absence of blood flow through a capillary it may gradually narrow until its lumen may permanently disappear —rather a retrogression in growth than contraction.

Nothing was seen which could be interpreted as active contraction of any of the veins or venules under observation.

Application of heat to the ear caused an extremely rapid flow of blood, the arteries, veins, and capillaries maintaining a uniform caliber. Cold applications caused persistent contraction of larger arteries with sluggish flow and slight widening of small arterioles, capillaries, and veins. With both heat and cold there was a marked degree of sticking of leukocytes to the walls of veins and venous capillaries, and, in the case of heat, extensive emigration of leukocytes through the endothelial wall.

Injection of adrenaline was followed by marked contraction of the arteries and arterioles at places where they were obviously provided with smooth-muscle cells, while capillaries and venules showed only slight, and apparently passive, changes in caliber. Contraction of arterioles was followed, after a few minutes, by a half-hour's dilatation.

Intravenous injection of histamine produced relaxation and widening of the small arteries and arterioles, coupled with diminution in blood flow. Capillaries and small veins showed no appreciable change in caliber.

Many of the interesting pictures to be seen in watching the circulation are described, most interesting of which are perhaps 'plasma skimming' (Krogh), platelet 'skimming,' and leukocyte 'skimming.'

Whether the new vessels under observation were supplied with nerves was not determined.

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BOOK REVIEW

THE SOCIAL LIFE OF MONKEYS AND APES. By S. Zuckerman. Harcourt, Brace Co., New York, 1922. 356 pp., 24 plates.

The author of this volume is anatomist to the Zoölogical Society of London and lecturer in physical anthropology in University College. Like his predecessor Sonntag, he has made intelligent use of the wealth of Primate material of the London Zoölogical Garden. Moreover, in keeping with the times, he has shown wider interests, having added physiology and psychology to his fields of investigation, and has considered the anthropological and the sociological implications of his findings and those of others. He has also studied, even though only for a brief period, one species of baboon, the Chacma of South Africa, in its native habitat and is, therefore, in a position to compare their behavior with that of the Hamadryas baboon of 'Monkey Hill' in the London Zoölogical Garden. The monkey is found to behave in captivity essentially as it does in the wild, but enforced leisure and the restricted quarters of captivity lead to more fighting which results in a high mortality, especially among the females over which most of the fighting takes place.

The more recent literature on the reproductive habits and social life of monkeys and apes in the wild, mostly the testimony of travelers, is reviewed in a useful way. Toward these data the author keeps an open mind while emphasizing the need of the greatest caution. His breadth of sympathy is, however, illustrated by his interest in the "trail of fantasy left by apes and monkeys in the old stories of explorers" as literature, entirely aside from what little of scientific value lies buried in them.

For the orientation of the general reader three chapters are devoted to the physiology of reproduction, in which field the author, partly in cooperation with A. S. Parkes, has made important contributions. The sexual cycle of mammals in general is presented briefly but accurately, the menstrual cycle in greater detail. First hand corroborative observations are made on a large number of monkey species, so that it is now safe to generalize and assume a cycle of about a calendar month for all Old World monkeys and apes.

The American workers, Corner and E. Allen, will be amused to learn that "the baboon is the only sub-human primate whose ovarian

cycle has been investigated in detail''—a statement made on the basis of a few autopsies of females that ended tragically on Monkey Hill and four non-pregnant, non-lactating females killed in South Africa! These six or eight cases likewise prompt the statement that ''no evidence was obtained that the baboon undergoes menstrual cycles without ovulation.'' By implication the author allows the reader to feel that perhaps the baboon differs from the rhesus monkey and resembles man in ovulating at each cycle. The records of the Carnegie Colony include over 300 menstrual cycles in which ovulation is known not to have taken place; yet it would be possible, on any day of the more favorable reproductive season of the year, to select six or eight females that ovulate regularly at that particular season.

With regard to the important problem of the time of ovulation in the menstrual cycle, Zuckerman estimates, on the basis of isolated fertile coitus during the stage of sexual swelling, that the egg is discharged at about the middle of the cycle. This agrees with published records of Corner, Allen, and Spiegel as well as 125 accurately determined cases of the Carnegie Rhesus Colony. Conception being determined in the baboon by the method of isolated coitus, the period of gestation in five cases was found to range from 154 to 180 (average 171) days, or about the same as thirty-eight rhesus gestations of the Carnegie Colony (149–180, average 165 days) and three Java macaques of Spiegel (160–170 days). Six lunar months would, therefore, seem to approximate the normal gestation period of the smaller Old World monkeys.

Zuckerman takes occasion again to present his theory of the homology of menstruation, a modificaation of Marshall's 'telescoping' theory, according to which the pro-oestrous degeneration of the dog endometrium and that following pseudopregnancy is viewed as telescoped into one. The theory seems futile and is certainly quite wrong, as it is based on phenomena that do not occur. Personal investigation has lead the reviewer to agree with Evans and Cole that Marshall and Jolly were in error in describing a pro-oestrous degeneration of the uterine mucosa in the bitch. Since the uterine epithelium remains intact also in the bleeding cow it is not likely that desquamation of the endometrium is to be regarded as an oestrous phenomenon, even though red blood cells do sometimes find their way into the uterine lumen during the stage of congestion.

Chapter IV contains an interesting if empirical classification of neuter animal societies in the anoestrum, male, female, or mixed, and the probable causes for the associations in the absence of sex attraction.

Under 'Periodicity' the breeding season of mammals in general and monkeys in particular is discussed. Though a commendable skepticism is professed and though the evidence for every species except man

and the rhesus monkey is extremely fragmentary, Zuckerman concludes that "monkeys and apes, like man, experience a smooth and uninterrupted sexual and reproductive life." This 'fact' is made the basis of theoretical discussion. Stress is laid on the analysis of twelve Chacma females killed in South Africa on May 4, 1930: four non-pregnant, but running ovulatory cycles; five in various stages of pregnancy; three lactating, the infants 2 to 4 months of age (estimated). The author ignores the fact that the reviewer had listed just such a group of fourteen rhesus females of the Carnegie Colony as of May 15, 1930, although the rhesus monkey exhibits a highly favorable season for conception from October to December, a highly unfavorable season in June, July, and August. This curve of fertility Zuckerman disposes of by defining breeding season as "the entire period in which all members of the species show sexual activity." Accordingly, since in our list two out of fifty-nine conceptions occurred in the June–August period, the rhesus monkey would be said to have no 'breeding season.' The challenge to explain the rhesus curve of fertility still remains, however; likewise the need for breeding data of other species comparable with those facts which the reviewer has brought together for M. rhesus.

Chapter X constitutes a well-considered excursion into comparative psychology. Conditioned and unconditioned sexual reactions are illustrated and discussed. The senses, particularly sight and touch, are considered in relation to arboreal life and to the use of the forefeet as hands. The author criticizes adversely the results of experiments on 'imitation,' holding that imitation has not been demonstrated. He favors what he wrongly considers Thorndike's conclusion that, while cats and dogs learn by trial and error, monkeys learn also in a qualitatively different way, namely by 'insight' (Koehler).

Two chapters are devoted to sexual behavior in relation to the oestrous or menstrual cycle. In rats and guinea-pigs, sex recognition depends purely upon the response of the receptive female, entirely without reference to sight, smell, or even an intact cerebral cortex. Visual and tactual stimuli are said to play a major rôle with the monkey. Sex color in some monkeys (e.g., rhesus), sex swelling in baboons and chimpanzees, are regarded as the chief stimuli to the male. However, inasmuch as some species of monkeys show neither sex color nor sex swellings, yet seem to behave characteristically, one wonders whether either is the cause of the male's heightened sex interest. Moreover, the sex skin of old rhesus females is chronically brilliant, as it always is during pregnancy and lactation. The subject of sex behavior of the monkey from the physiological standpoint requires complete study under experimental conditions.

As a background for discussing the organization of monkey societies the author shows that the reproductive needs of the species form the mainspring that keeps societies together, a viewpoint thus far exploited only by Howard for birds, and by Malinowski and Briffault with respect to human society. Heterosexual attraction, with its physiological causation, determines social grouping. This, combined with 'dominance' of the leading male or males, determines the harem, the essential social element. The monkey societies are all polygynous. Even the horde is due to sex attraction, the females attracting even the inferior males, the bachelors. No female is unattached.

The author makes much of the phenomenon of dominance, which he does not define but illustrates by numerous examples. The males of Old World monkeys, always much larger than the females, 'dominate' those that they acquire and defend; and they likewise dominate the less virile, the submissive males. The weaker individuals demonstrate submission by 'presenting' sexually. The weaker males do this in exactly the same way as the females. Fear and flight reactions, Zuckerman theorizes, have metamorphosed into sexual presentation as a method of defense and finally as a means of obtaining food. This Hamilton calls 'sexual prostitution,' a term justified if results are considered rather than the origin of the habit or an attempt to interpret the monkey's mental processes.

Further comparative studies will show whether or not dominance plays the important rôle in all Primates that Zuckerman attributes to it; for the baboon would seem to be extreme both as to the degree of sexual dimorphism as well as the expression of male dominance.

What is innate, what conditioned in the behavior of the monkey can only be determined by Stone's method, used with rats, of isolating individuals from birth—truly a long drawn-out and expensive experiment in the monkey, but well worth while. The problem likewise obtrudes itself in the case of man, for whom "there is no state of nature," whose cultural inheritance, determined by oral and written speech, greatly modified those elements of behavior that are due to physical (physiological) causes. As to how far observations on the monkey are applicable to the interpretation of human behavior the author reacts pessimistically. "Certainly," he says, "if the study of human behavior itself will not reveal man's 'basic instincts and impulses,' comparison with the lives of other organisms is futile." It is dangerous to underrate the differences between man and his Primate contemporaries; yet the gap is bridgeable and on the whole "behavior common to all monkeys and apes can be regarded as representing a social level through which man once passed in the pre-human stages of development."

The chapters on mutual aid should be read by all familiar with Kropotkin's conclusions.

After writing the criticisms voiced in the foregoing paragraphs the reviewer hastens to state that the virtues of the volume far outweigh its few sins of commission or omission. Zuckerman has produced a work indispensable to the specialist and at the same time of interest to the layman. It is written in a smooth, vivid, and imaginative style and with a refreshing enthusiasm, which more than compensates for the rather loose organization and the consequent repetitions, which are, however, hardly apparent at the first reading. The bibliography of 402 titles is to be appreciated. The volume is a fit companion to Yerkes and Yerkes' "The Great Apes."

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